GENETIC DEAFNESS

Special reference to branchial arch syndromes

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Contents

General Introduction

Research into the causes of hereditary hearing loss and hereditary deafness was initiated in the second half of the nineteenth century. In this period, the first reports appeared on a number of fairly common hereditary syndromes with hearing loss as one of the characteristic symptoms.
For example, in 1858, the German ophthalmologist Albrecht von Graefe was the first to describe the occurrence of retinitis pigmentosa and congenital deafness in three affected brothers.[1]
Three years later Liebreich recognised this condition during a study on deaf persons in the Jewish population of Berlin and he drew attention to consanguinity between the parents of the affected individuals.[2] In 1907, Hammerschlag discovered a high frequency of the disorder among the Jewish community in Vienna.[3] This was followed in 1914 by an English description by the Scottish ophthalmologist Charles Usher from Aberdeen.[4] Usher also mentioned the condition in his Bowman Lecture in 1935 which was entitled, "On a few hereditary eye affections". As a consequence, his name became an eponym for the syndrome, especially in the ophthalmological literature.
Mandibulo-facial dysostosis or Treacher Collins syndrome, which forms part of this thesis, was described for the first time - although incompletely - by Thomson in 1847 and by Berry in 1889.[5,6] The description made by Edward Treacher Collins from London in 1900 on the basis of two cases, was also incomplete, but he was awarded the honour of his name remaining associated with this syndrome in the English medical literature.[7] He had been given his mother's maiden name, Treacher, as a christian name and in accordance with the customs at that time, he used it as a double-barrelled surname without a hyphen.
In 1949 the Swiss ophthalmologist Adolphe Franceschetti and the Swiss medical geneticist David Klein, both from Geneva, published the first extensive account of mandibulo-facial dysostosis by collecting all the available clinical material, including the doctoral thesis by Zwahlen (1944) and an article based on the thesis published by Franceschetti and Zwahlen (1944).[8,9] In the French literature the names of Franceschetti and Klein became an eponym for this syndrome.
Another example of a syndrome which was first described in the nineteenth century, is the Branchio-Oto-Renal syndrome, which also forms part of this thesis. It appeared in the literature for the first time in the form of a case report by Heusinger. He presented a seven-year-old girl and drew attention to slight dysplasia of the pinna, a preauricular sinus, severe hearing loss and cervical fistulae; the latter symptom had prompted him to publish his findings.[10] In 1878, the famous surgeon Sir James Paget

from London described the syndrome - in almost its full extent - after discovering it in two generations of one family.[11]
Although physicians had known for several centuries that congenital deafness in a young child could also occur in his or her siblings, the first systematic study on the causes of congenital deafness was conducted by Sir William Wilde from Dublin in 1853.[12,13] He recognised heredity as the cause of congenital deafness and also concluded that consanguinity between the parents increased the risk of this disorder.
In 1882, Politzer wrote the following in his textbook on otology on the basis of conclusions from other studies: "..... The most frequent causes of congenital deafness are: heredity, including direct transmission from the parents as well as indirect transmission from fore fathers and marriage between bloodrelations.....".[14,15]
It took until the beginning of the twentieth century, however, for Mendel's laws (1865) to be recognised as an explanation for heredity.[16]
In the first half of the twentieth century, studies on the causes of deafness were continued on a large scale and several impressively large isolates were published in detail, including autosomal recessive hereditary deafness.[17,18] Many of the investigations at that time were devoted to the study of deafness in various types of animal.[17]
Knowledge about the syndromal and non-syndromal forms of hereditary hearing loss has increased strongly, particularly in the second half of this century. The more common syndromes were described repeatedly, in great detail, with special attention to the degree of penetrance of the syndrome and the degree of expression of the separate symptoms. On the basis of heredity, the type of hearing loss and other audiometric characteristics, it was possible to describe a series of separate non-syndromal hereditary forms of hearing loss. In this period, the first overviews appeared of the many (and ever increasing number of) hereditary syndromes with deafness as one of the symptoms.[19-29]

In Western Europe, the prevalence of early childhood deafness (average hearing level in the best ear of > 25 dB) is estimated to be 1 in 750 to 1000 births.[30,31] It is likely that there is a genetic cause in over 50% of these cases.[23,25,32-46]
In the nineteen seventies, more than 150 (non-)syndromal hereditary forms of hearing loss had been documented and the number has increased since then.[47,48] In a few of these generally autosomal hereditary diseases, gene-linkage studies have very recently led to gene localisation (Table I).[49,50] This thesis contains successful contributions of gene-linkage studies for two of these syndromes: the BOR syndrome and the Treacher Collins syndrome. For twelve hereditary syndromes with hearing loss or deafness as one of the symptoms, a gene has actually been isolated recently. Continuing studies and definitions of other (non-)syndromal forms of hereditary deafness are necessary to enable the genotypic characterisation of other monogenic abnormalities.

In this thesis, a number of phenotypic and/or genotypic aspects of one of the non-syndromal autosomal recessive hereditary forms of congenital deafness and three other syndromal autosomal dominant forms of congenital deafness were subjected to further study.
The study questions are formulated below, in the light of these separate syndromes.

Table I: *Chromosomal location of genes causing inherited diseases with hearing loss.*

Autosomal dominant	Linkage	MIM#	Gene cloned	References
Neurofibromatosis type II	22q	101000	+	Rouleau, 1993[61]
Branchio-oto-renal syndrome	8q	113650	-	Kumar, 1992[62]
Stickler syndrome	12q	120140	+	Knowlton, 1989[63]
Osteogenesis imperfecta	17q	120150	+	McKusick, 1992[48]
Osteogenesis imperfecta	7q	120160	+	McKusick, 1992[48]
Hereditary low frequency hearing loss	5q	124900	-	León, 1992[64]
Treacher Collins syndrome	5q	154500	-	Dixon, 1993[65]
Neurofibromatosis type I	17q	162200	+	Wallace, 1990[66]
Facioscapulohumeral muscular dystrophy	4q	158900	-	Wymenga, 1992[67]
Piebald trait	4q	172800	+	Spritz, 1993[68]
Rieger syndrome	4q	180500	-	Shiang, 1987[69]
Waardenburg syndrome type I	2q	193500	+	Pasteris, 1993[70]
Autosomal recessive				
Hurler syndrome	4p	252800	+	Scott, 1990[71]
Usher syndrome type I	14q	276900	-	Kaplan, 1991[72]
	11q		-	Kimberling, 1992[73]
Usher syndrome type II	1q	276901	-	Kimberling, 1990[74]
X-linked				
Albinism-deafness syndrome	Xq	300700	-	Shiloh, 1990[75]
Alport syndrome	Xq	303630	+	Barker, 1990[76]
Deafness, conductive, with stapes fixation (and stapes gusher)	Xq	304400	-	Brunner, 1988[77]
Hunter syndrome	Xq	309900	+	Le Guern, 1990[78]
Norrie disease	Xp	310600	+	Berger, 1992[79]
Otopalatodigital syndrome type I	Xq	311300	-	Biancalana, 1991[80]
Pelizaeus-Merzbacher disease	Xq	312080	+	Raskind, 1991[81]

Non-syndromal autosomal recessive profound childhood deafness

Although studies have shown that the carriers of two X-linked hereditary syndromes with hearing loss can be detected using audiometry, the same has been suggested for autosomal recessive non-syndromal hereditary congenital deafness, but it has not yet been confirmed.[35,51-53]

By conducting a large familial examination we were able to answer the question of whether carrier detection is possible via clinical examination alone of this Dutch form of early childhood deafness. In addition, a conclusion could be drawn regarding the expression of this hearing loss c.q. deafness.

With the aid of this family and through an accurate description of the form of non-syndromal autosomal recessive deafness present in this family, it was possible to start gene-linkage studies on the syndrome in cooperation with the Department of Human genetics, University Hospital Nijmegen, the Netherlands.

The Branchio-Oto-Renal (BOR) syndrome

In the period from 1977 to 1981, Cremers and Marres, Cremers and Fikkers van Noord, Cremers et al. and Widdershoven et al. described the phenotypic aspects of the BOR syndrome in several Dutch families with this syndrome.[54-57] Both the frequency of the symptoms and the variation in expression were discussed in detail. Fraser et al. (1978) showed that renal abnormalities also formed part of the syndrome and since then it has gradually been accepted that the branchio-oto-renal syndrome and the branchio-oto syndrome, also known as the earpits deafness syndrome, were the same syndrome.[58] The renal abnormalities were not noticed in the earlier studies so no systematic renal tests were performed.
In Nijmegen, the results of reconstructive ear surgery in patients with this syndrome were disappointing.[56] In 1981, also in Nijmegen, the first attempt was made to perform gene-linkage studies to isolate the affected chromosome. This was the first time that the results of gene-linkage studies were published on a syndrome with hereditary deafness.[55]
Owing to the fact that important data on the degree of penetrance and the degree of expression of the BOR syndrome were already available in the literature, we continued the research by initiating gene-linkage studies for this syndrome. In 1987, we came into contact with Prof. W.J. Kimberling, Center for Hereditary Communication Disorders, Boys Town, Omaha, USA for the first time. For the purpose of these studies, blood samples were taken from the largest Dutch family mentioned in the references above so that gene-linkage studies could be repeated in the USA.
Despite the earlier disappointing results of reconstructive ear surgery obtained specifically in the patients with this syndrome, we continued to search for new methods and techniques to improve the congenital hearing loss using surgery. The results of a new type of reconstructive ear surgery is described.

A branchial arch syndrome not described before

Within the framework of earlier clinical studies on the BOR syndrome and an application for genetic counselling from another family because of symptoms suspicious of the BOR syndrome, we noticed that several of the members of this (as yet not fully examined) family did not have the cervical fistulae which form part of the BOR syndrome.
A study was initiated in which a thorough examination was made of this family to see whether they were suffering from the BOR syndrome and, if possible, to use their data in support of the gene-linkage studies on the BOR syndrome, or whether they were suffering from a syndrome which resembles the BOR syndrome.
This led to the discovery of a new syndrome. The otological and audiological aspects of this syndrome were investigated separately. Blood samples were also taken from this family who, at that time, had an unknown syndrome. These were sent to the Center for Hereditary Communication Disorders, Boys Town, Omaha, USA, so that as soon as gene-linkage had been accomplished for the BOR syndrome, it could be investigated whether this syndrome could be linked to the same part of the same chromosome.

The Treacher Collins syndrome

Another serious branchial arch syndrome is the Treacher Collins syndrome. Although this syndrome has been known for more than a century, it is noticeable that there seem to be many spontaneous mutations and that relatively few large families with affected members over three or four generations have been described in the literature.[25] Van Rijn mentioned one case of non-penetrance in his thesis.[59]
Gene-linkage studies were performed in Manchester in which this syndrome was linked to chromosome 5q. We received a positive answer to a request to combine our (research) forces with those of Dixon and his team from Manchester in support of his gene-linkage studies. Two fairly large families were approached and invited to participate in a clinical syndromal study. At the same time, blood samples were taken from the family members to further the gene-linkage studies. The first intention was to find out more about the degree of penetrance and the variation in expression and, with the aid of gene-linkage studies, to establish whether it was not unusual for a person affected by the syndrome to show only slight symptoms or none at all.
Reconstructive middle ear surgery and reconstructive surgery because of aural atresia are not always successful in patients with the Treacher Collins syndrome. Therefore, an overview of the literature was made, supplemented by our own surgical results, to find an answer to the question of whether attempts to perform reconstructive surgery on isolated congenital anomalies of the middle ear and/or attempts to perform reconstructive surgery on congenital aural atresia in patients with this syndrome, meet with sufficient success in selected cases. A new alternative method is the surgical fitting of a percutaneous bone anchored hearing aid.[60] The extent to which this method is successful in patients with the Treacher Collins syndrome is discussed.

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Chapter One

Non-syndromal Genetic Deafness

1.1 Non-syndromal Profound Genetic Deafness in Childhood*

Abstract

About one-half of children with profound deafness have an autosomal recessive or autosomal dominant inherited type of deafness. The X-linked inherited types of deafness are rare. About one out of three profoundly deaf children has an autosomal recessive form of inherited deafness. At sometime during their life a syndromal diagnosis can be made in one out of four cases with an autosomal recessive form of deafness. Therefore in about 25% of all the children with profound deafness, a nonsyndromal autosomal recessive type of genetic deafness will be involved. It is still not clear how many different genes are responsible for this. The more severe the deafness in a child, the greater the chance that an autosomal recessive etiology is involved.
The autosomal dominant inherited types of deafness are significantly more frequent in cases where the hearing loss in the best ear is less than 80-90 dB. About one-half of the autosomal dominant inherited cases show a classical syndromal type of deafness based on clinical features. In the other half, some audiometrically recognizable types of deafness can be diagnosed after an autosomal dominant pattern of inheritance has been established.
Additional genetic knowledge based on gene-linkage studies is needed to provide better tools for the more accurate diagnosis of genetic etiology in a profoundly deaf child.
Adequate pedigrees are quite rare and such pedigrees are expected to become even more scarce as a result of a diminishing ratio of consanguineous marriages. It is necessary to start gene-linkage studies in these existing pedigrees to trace the genes responsible for this nonsyndromal type of profound genetic deafness in childhood.

Introduction

The incidence of childhood deafness, defined as a hearing loss of 50 dB in the best ear, is one in one thousand children in the West European countries.[1] If we redefine this deafness as a loss of more than 30 dB in the best ear, then the incidence becomes one in seven hundred children. Therefore, we are not talking about a rare disorder.

*C.W.R.J. Cremers, H.A.M. Marres and P.M. van Rijn. Genetics of Hearing Impairment. Annals of the New York Academy of Sciences 1991; 630: 191-196.

Table I: *Causes of Early Childhood Hearing Impairment and Deafness (from Nine Studies)[a]. [a]Deaf is mean hearing loss 0.5, 1.0, 2.0 kHz of the best ear > 40 dB, [b]No data, [c]Insufficient data*

Etiology	Huizing (n=100; hearing impaired/ deaf)	Fraser (n=2355; hearing impaired/ deaf)	Fraser (n=3535; hearing impaired/ deaf)	Cremers (n=60; deaf)	Newton (n=111; hearing loss >25 dB)	Holten (n=94; hearing loss >65 dB)	Kankunen (n=179; hearing loss >25 dB)	Parving (n=117; hearing loss >35 dB)	van Rijn (n=162; hearing loss >35 dB)	These Studies Together (excluding Fraser)[4]
Hereditary	20	32	49.6	37	25	33	55	48	40	20-55
Autosomal dominant	8	c	15.2	2	12	c	b	b	14	2-14
Autosomal recessive	12	c	32.7	35	13	c	b	b	23	12-35
X-linked	b	c	1.7	b	c	c	b	b	1	1
Acquired	56	32	50.4	38	32	41 7	29	42	27	27-56
Meningitis	6	17	c	13	4		7	3	6	3-17
Rubella	3	6	c	5	11	15	5	19	6	3-19
Kernicterus	7	4	c	15	14	15	1	14	6	1-19
Asphyxia	9	1	c	2			1		2	1-15
Otitis	26	1	c	b	b	1	6	3	4	1-26
Others	5	3	c	3	2	3	9	3	4	2- 9
Unknown	24	36	b	25	43	26	16	10	34	10-43

So far, nearly all the research into the causes of childhood deafness has been carried out in retrospect, except for a few studies that examine one cause, for example, meningitis. Studies that aim at compiling a list of causes of childhood deafness are usually referred to as school studies. An overview of a number of important Western European School studies is shown in Table I.[2-11] Only one of these studies examined children with a hearing loss of over 90 dB. The majority of studies only mention the lower limit of the hearing loss as a criterion for inclusion in the study. The only criterion in Fraser's study was that the children had to be pupils at one of the schools for hearing impaired children that they visited.
In a recent school study, van Rijn was the first to relate the causes he found to the extent of the hearing loss.[11] He presented his data in an easily understandable manner,in steps of 10 dB, in order to demonstrate any possible correlations with the cause of hearing impairment.
Unfortunately, the study designs employed in many Western European and other publications vary considerably. This is also true of the quality of the medical files available for retrospective research, not to mention the differences in quality of the data documented. Very few studies applied regular genealogical pedigree research to examine the value of this approach.
Despite these shortcomings, the results of these studies do provide us with some insight into the prevalence of the various causes of childhood deafness. In order to review these results, it is necessary to bear in mind that in Fraser's final results, the children with an unknown etiology were distributed among the various causes he described.[4]

Incidence of genetic deafness

X-linked Deafness
The incidence of X-recessive and X-dominant types of inherited deafness in children is estimated to be about 1%. The most familiar clinical pictures of these inherited hearing losses form a symptom complex, for example, the stapes gusher syndrome, Norrie's syndrome, and the Alport syndrome.[12-14]
Only an occasional report has appeared on a nonsyndromal X-linked type of inherited deafness.[15] In view of the low prevalence, we will not discuss the nonsyndromal X-linked type of inherited deafness any further.

Autosomal Dominant Inherited Deafness
Studies have shown that the incidence of autosomal dominant inherited deafness depends on the extent of the hearing loss. In cases where the hearing loss in the best ear exceeds 70 dB, an autosomal dominant inherited type of deafness is fairly uncommon.[11] This is understandable, because over the years, severely deaf people will have had poor marriage prospects and will have found it difficult to find a partner and procreate; thus negative selection has taken place. In the case of autosomal dominant inherited deafness, it is particularly the changing penetration that accounts for the fact that the disorder is passed on by someone who is less seriously affected.
When the above is taken into consideration, it is not unreasonable that 15% of hearing impaired children suffer from an autosomal dominant inherited type of deafness.

In half of them, a syndromal diagnosis can be made, but not in the other half. Therefore in order to establish an autosomal dominant inherited type of deafness in the latter half, family studies must be performed, preferably over three generations. Furthermore, if some of the audiometric curves obtained from the family members show a progressive course, it will be possible to recognize certain specific forms of autosomal dominant inherited deafness. Well-known examples include midfrequency deafness and progressive high-tone inner ear deafness.[15]
In this way, careful examination of the family members can lead to the detection of an individual case of autosomal dominant inherited deafness, even when there are no syndromal symptoms.

Autosomal Recessive Inherited Deafness
In order to make the diagnosis of autosomal recessive inherited childhood deafness if there is no evidence of a syndromal diagnosis, several points should be kept in mind: it is necessary for at least one sister or brother, but preferably more siblings, to be affected and no indications of environmental causes. In addition, the existence of consanguinity between the parents is a factor that is as powerful as the observation of another affected sib in favor of an autosomal recessive etiology.
If these criteria are to be fulfilled, the family must comprise more than one child, which will probably mean that the family has been completed. By this time, the propositus will most likely be an adolescent. The age of the affected child at the time of the etiology study therefore influences the frequency with which the diagnosis autosomal recessive inherited deafness can be made.
The same effect is also encountered via a similar route in the well-known syndromal forms of autosomal recessive inherited deafness, such as the Usher syndrome types I and II and the Pendred syndrome, because the diagnosis is not often made before the age of 10 or until adolescence.
If there are no clear indications for a different etiology and it is not possible to demonstrate an autosomal recessive inherited type of deafness on the basis of the above-mentioned criteria, then the etiology will have to be documented as unknown.
In the study by van Rijn, he did not find that autosomal recessive inherited deafness was significantly more prevalent in the group of persons with a hearing loss of above 80 dB compared with those with less than 80 dB loss.[11]
On the other hand, Newton has made it credible that the chance that autosomal recessive inheritance is involved in the group with an unknwon etiology increases as the severity of the hearing loss increases.[7]
Table 1 shows that the diagnosis of an autosomal recessive inherited type of deafness is made in 13-35%. In reality this percentage will be much higher because in the group with an unknown etiology, a great many will inevitably be suffering from autosomal recessive inherited deafness.
In the light of the above, we can see that it is impossible to demonstrate the existence of autosomal recessive inherited deafness in the first, young, hearing-impaired child of parents with good hearing who are not blood relations. If extensive retrospective research has failed to uncover sufficient evidence for a different etiology we personally assume on the basis of empirical data that the likelihood that the child has autosomal recessive inherited deafness is about 80%.[16]
In the literature also a percentage for this recurrence risk has been reported.

The Perspective

Genetic counseling in the case of a young, hearing-impaired child is often unsatisfactory because the suspicion of an autosomal recessive inherited etiology is mainly based on the exclusion of the other inherited or acquired causes.
This situation can only be improved by the introduction of diagnostic tests at genetic level. This means that we need the cooperation of large families who have undergone extensive clinical-genetic investigation, in whom several families and several family members have proven autosomal recessive inherited deafness without syndromal characteristics. Such families have only occasionally been described in the literature.[17-23] However, we may assume that there are more. It is possible to find such pedigrees in religious or geographically isolated families, but as people have become more mobile, the ratio of consanguineous marriages has dropped and isolated places have become more accessible.
In view of the recent availability of new gene-linkage tests, the best approach would be to reexamine the sufferers and nonsufferes in these pedigrees, in order to start contiguous gene-linkage studies. We estimate the incidence of this nonsyndromal autosomal recessive inherited deafness to be one in three or four thousand. These figures underline the need for very active participation in such research.

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1.2 Autosomal Recessive Non-syndromal Profound Childhood Deafness in a Large Pedigree*

Abstract

Nonsyndromal autosomal recessive profound childhood deafness will affect about one in 4000 children in western Europe. A nonsyndromal autosomal recessive type of profound childhood deafness was thought to be the cause of deafness in at least eight and probably 12 children from a large family with various consanguineous matings and other family interrelations. Audiograms of all affected deaf children showed a profound childhood deafness with only a very slight variation. Audiometric examinations, such as pure-tone audiometry, high-frequency audiometry, stapedial reflexes, and Békésy audiometry, of ten obligate or presumed carriers did not show any significant findings that would allow identification of carriers of this autosomal recessive gene. Families like this one seem to be very rare. Large clinically well-studied families like this one are indispensable for gene-linkage studies of nonsyndromal autosomal recessive types of profound childhood deafness. Such studies should make it possible to trace the origin of these types of childhood deafness at an early age. In consequence, carrier detection should also become available.

Introduction

At least 1/1000 children in western Europe proves to be deaf during early childhood.[1] In approximately half of these cases, a hereditary cause seems to be probable.[2-9] The mode of inheritance is usually autosomal recessive; autosomal dominant is less frequent. Sex-linked, inherited deafness is extremely rare. In no more than one fourth of children with an autosomal recessive form of inherited deafness, a syndromal illness complex can be identified. It therefore follows that one in 4000 children in western Europe suffers from a nonsyndromal autosomal form of profound childhood deafness. It is probable that several different genes are responsible.[10,11]
In the near future, gene-linkage studies could contribute to the recognition of these separate clinical-genetic forms of deafness in the young child. In turn, this could con-

*Henri AM Marres, Cor WRJ Cremers. Archives of Otolaryngology - Head & Neck Surgery 1989; 115: 591-595.

tribute to the establishment of an autosomal recessive mode of inheritance in many young children in whom, at present, the cause frequently remains undetermined. Such studies may also allow future identification of carriers of that autosomal recessive gene. Prenatal diagnosis would also become possible.
Such an increase in knowledge would represent a tremendous advance, especially for genetic counseling. The performance of gene-linkage studies of this kind would require the cooperation of a very large family with a moderately large number of sufferers of the same type of autosomal recessive inherited childhood deafness.
To identify such a family, we renewed our contacts with two family units with a total of 18 children, six of whom had been deaf since childhood.[3] The mothers of these two families are sisters. The parents of both families share a direct bloodline back to the same ancestral couple. Reports that there might be further cases of profound childhood deafness among other members of the family was, for us, reason enough to begin new, extensive family studies.
The object of the study was to investigate the range of types of hearing loss among the deaf subjects and to investigate the possibility of recognizing the carriers of this autosomal recessive gene by means of audiometry.

Family and Methods

A genealogical study was performed to establish the relationship between the many deaf people in this family and their direct relatives. The pedigree is presented in Figure 1. Information regarding the cause of deafness in the six children from the first two family units known to us (subjects IX_{15}, IX_{17}, IX_{26}, IX_{27}, IX_{28}, and IX_{29}) has already been published by one of us (C.W.R.J.C.).[3,4] In all of these subjects, the medical history gave no indication at all for the possibility of an acquired form of deafness. Ophthalmological examination, especially for the exclusion of Usher's syndrome and rubella retinopathy, showed no abnormalities. Results of a general physical examination were also normal.
A potassium perchlorate test using labeled iodine 123 for the identification of Pendred's syndrome was normal in all six subjects.[12] Owing to the blood relationship between the first set of parents that could be traced back to the same ancestral couple, the family relationship between the two mothers (siblings), the fact that in each of these two families more than one child was deaf, and also the previously mentioned medical findings, a diagnosis was made of a nonsyndromal form of autosomal recessive profound childhood deafness.[3]
A general medical history and a more specific history aimed at identifying the possible causes of deafness were taken from all hearing and deaf members of the family. These histories were also taken once again from the previously investigated subjects. All subjects underwent an otological examination and were scrutinized for the presence of syndromal features. An additional ophthalmological examination was performed on subjects IX_{33}, X_1, X_2, and X_7. An audiogram was made in a soundproof cabin (JAC) using a clinical audiometer (Madsen OB 822) on a total of 58 subjects. This was performed partially to be able to evaluate possible variation in the severity of hearing loss. Further audiometric examination, including stapedial reflex measurements, Békésy audiometry, and high-frequency audiometry, were performed on a to-

tal of ten obligate (subjects $VIII_{10}$, $VIII_{11}$, IX_{10}, IX_{11}, X_5, and X_6) or probable (subjects $VIII_{13}$, $VIII_{14}$, IX_{35}, and IX_{36}) carriers of this autosomal recessive gene, ie, eight of ten parents of the deaf subjects and the two normal-hearing children of one of the deaf subjects.

Results

All 58 subjects cooperated in this study (Figure 1). It was noteworthy that there were three family units with a blood relationship between the parents, and that each of these families contained more than one deaf child. The pedigree contains a total of 16 deaf subjects. Four of these subjects (IX_1, IX_{32}, IX_{37}, and X_{10}), although having been deaf or hard of hearing from early childhood, have a medical history such that an autosomal recessive inheritance is an unlikely cause for their loss of hearing. There is no doubt that the deafness in eight of the subjects (IX_{15}, IX_{17}, IX_{26}, IX_{27}, IX_{28}, IX_{29}, X_1, and X_2), from the three family units mentioned above in which there is a blood relationship between the parents, is autosomal recessively inherited. With regard to the remaing four subjects ($VIII_1$, IX_{33}, X_3, and X_7), based on the absence of any other possible explanation for their deafness and their family relationship with so many other deaf subjects, it is highly likely, although not certain, that the same autosomal recessively inherited form of deafness is involved.
The pure-tone audiograms from the eight subjects who were certainly suffering from the same form of autosomal recessive profound childhood deafness show little

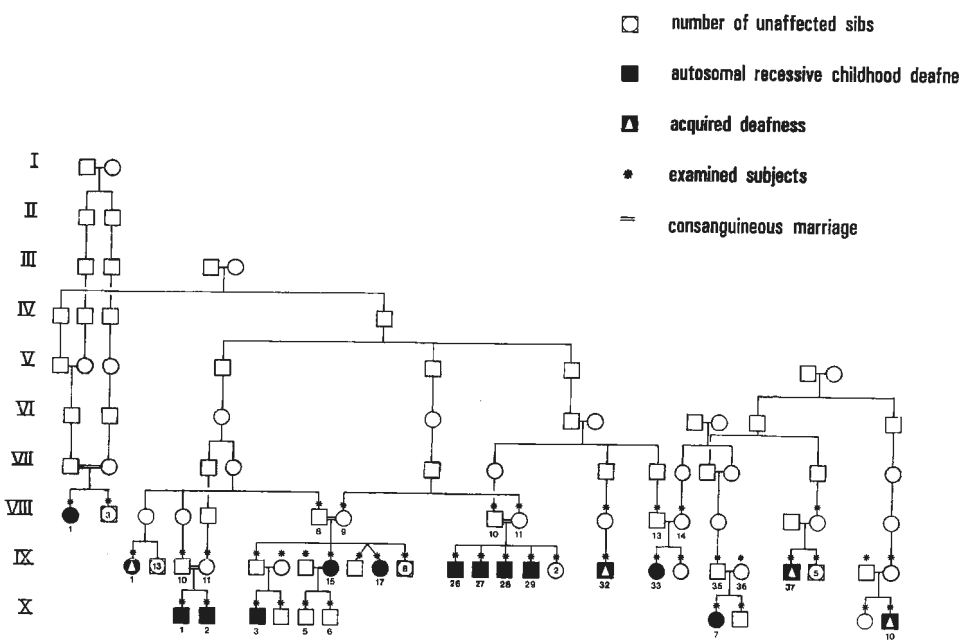

Figure 1: *Unique pedigree with nonsyndromal autosomal recessive profound-childhood deafness.*

variation (Table I and Figure 2). Comparison with previous audiograms made during a period of up to 32 years shows that this not a progressive form of deafness.
With the exception of the heard-of-hearing son (X_3) of one of the siblings, little variation was found in the hearing loss of those subjects who were probably suffering from the same form of deafness and the other deaf subjects (Table I).
The pure-tone audiograms from the 12 obligate or probable carriers (Table II) showed slight abnormalities in eight subjects. In three of these subjects, the history and the typical form of the audiogram indicated noise damage ($VIII_{10}$, $VIII_{11}$, and IX_{35}). Subjects $VIII_{14}$ and IX_{36} showed an unexplained slight high-frequency sensorineural hearing loss. Subject $VIII_{13}$ had an unexplained slight hearing loss for lower frequencies. In two olders subjects, the high-frequency loss was in keeping with presbyacusis ($VIII_8$ and $VIII_9$).
Both impedance measurements and registration of contralaterally stimulated stapedial reflexes in these subjects showed no abnormalities of the type described by Anderson and Wedenberg.[13,14] Raised reflex thresholds were not found.
The high-frequency audiometry findings in these ten obligate or probable carriers are shown in Table III. The findings in normal-hearing agematched control groups, with corrections for the apparatus used, are shown in Table III as reference points.[15] We did not consider the results to be abnormal, taking into account the above-mentioned explanations for the slight changes found in the ordinary pure-tone audiograms of six of the obligate or probable carriers.

Table I: *Pure Tone Thresholds in 16 Deaf Subjects. The top of 12 cases are most probably subjects who all suffered from the same form of nonsyndromal autosomal recessive profound childhood deafness; bottom four cases, subjects who were presumed to suffer from acquired forms of deafness. Values are given as pure-tone thresholds, right ear/left ear, in decibels throughout.*

	Frequency, Hz					
Case	250	500	1000	2000	4000	8000
Hereditary Deafness						
IX_{15}	85/80	90/90	100/100	100/95	115	115
IX_{17}	65/65	80/80	95/90	105/100	115	115
IX_{26}	65/70	80/90	100/110	115/115	115	115
IX_{27}	100/90	105/100	115/115	115	115	115
IX_{28}	70/70	90/90	105/110	115/115	115	115
IX_{29}	80/70	90/85	105/100	115/105	115/100	115/100
X_1	80/85	95/95	110/105	110/110	115	115
X_2	75/75	85/85	95/95	95/100	100/-	85/-
$VIII_1$	115/85	115/90	115/90	115/95	115	115
IX_{33}	115/100	115	115	115	115	115
X_3	15/15	20/30	70/50	65/50	70/20	60/10
X_7	90/85	95/95	110/110	110/110	115/95	100/100
Acquired Deafness						
IX_1	60/80	55/80	60/65	50/55	60/70	85/90
IX_{32}	10/10	15/15	25/45	55/55	85/80	55/55
IX_{37}	115/75	115/80	115/80	115/75	115/80	115/85
X_{10}	85/85	95/100	110/105	110/115	115	115

Table II: *Pure-Tone Audiometry in Obligate and Probable Carriers of Same Autosomal Recessive Gene for Form of Autosomal Recessive Profound Childhood Deafness. Values are given as pure-tone thresholds, right ear/left ear, in decibels throughout.*

		Frequency, Hz					
	Age, y	250	500	1000	2000	4000	8000
Carriers							
$VIII_8$	68	25/25	20/25	20/30	40/55	65/70	85/80
$VIII_9$	67	30/30	30/30	25/20	30/25	40/40	85/80
$VIII_{10}$	58	5/5	5/5	15/15	10/15	30/40	10/35
$VIII_{11}$	55	20/35	20/25	15/15	10/10	20/20	10/25
IX_{10}	43	5/10	10/10	10/5	0/5	20/15	10/15
IX_{11}	40	10/5	5/5	5/5	5/0	10/15	15/10
X_5	16	10/5	5/5	5/5	5/5	5/5	10/5
X_6	13	15/15	10/15	5/10	-5/-5	0/5	10/20
Presumed carriers							
$VIII_{13}$	41	30/20	25/20	20/15	0/0	20/20	25/30
$VIII_{14}$	41	5/10	5/10	10/15	15/10	15/20	30/35
IX_{35}	38	15/10	0/5	5/5	5/5	30/25	10/20
IX_{36}	37	10/10	5/5	0/0	5/0	20/5	25/15

The four obligate carriers with normal audiograms also showed normal results in Békésy audiometry. In the other two carriers, changes were found that were in agreement with those described earlier in the pure-tone audiogram. This also applied to the four probable carriers. None of them showed a "dip" of 20 dB or more over an octave, as was described by Anderson and Wedenberg[13,14] as being characteristic of obligate carriers of nonsyndromal autosomal recessive profound deafness.

Comments

It is known that in sex-linked inherited diseases, the female carrier with one normal and one abnormal X chromosome frequently shows mild clinical symptoms. In such diseases that involve deafness, eg, X-recessive Norrie's disease, X-recessive progressive mixed deafness, and X-dominant Alport's syndrome, abnormal audiograms have been described in the female carriers. Anderson and Wedenberg, using Békésy audiometry, described a dip around 1500 to 2000 Hz in a group of parents of children who were presumed to suffer from an autosomal recessive profound childhood deafness.[13,14,16,17] An abnormally raised threshold for the induced stapedial reflex was also found in a large group. The cause of the deafness, however, was frequently not satisfactorily demonstrated. Furthermore, the study involved children from many different family units without blood relationships. It is therefore highly unlikely that all these subjects were affected by the same form of genetically determined deafness. Newton and van Rijn, in their independent but comparable studies of the causes of deafness that dit not, however, employ Békésy audiometry, could not confirm the findings of Anderson and Wedenberg.[7,9,13,14] In their earlier studies, Wildervanck and

Table III: *High-Frequency Audiometry (8 to 20 kHz) in Obligate and Probable Carriers. The normal values (N) for each age group are given as reference points; the values for high frequency audiograms, right ear/left ear, are given in decibles throughout.*
Minus sign (-) preceding numbers indicates this is a better value than the standard. The minus signs separated by a virgule indicate no real value can be measured since this value in this patient is beyond the range of the audiometer.

Carrier	8	N	10	N	12	N	14	N	16	N	18	N	20	N
Obligate														
$VIII_{10}$	-10/-10	40	0/35	60	25/35	80	20/30	100	5/5	115	-/-	120	-/-	120
$VIII_{11}$	10/0	40	5/5	60	15/25	80	20/20	100	5/5	115	-/-	120	-/-	120
IX_{10}	-10/-5	30	-15/-15	45	-20/0	60	-15/20	85	10/10	105	0/0	115	-5/-5	120
IX_{11}	0/5	20	10/0	25	- 5/-5	35	0/30	60	20/30	85	10/15	105	5/5	115
X_5	10/-5	15	15/-5	15	15/-5	25	0/-5	30	-10/-10	55	15/5	75	0/0	105
X_6	10/15	15	15/15	15	5/20	25	15/30	30	25/30	55	40/40	75	15/15	105
Probable														
$VIII_{13}$	15/25	30	10/15	45	5/5	60	30/10	85	15/15	105	5/5	115	0/0	120
$VIII_{14}$	0/20	30	0/30	45	0/30	60	5/25	85	15/15	105	5/5	115	0/0	120
IX_{35}	0/10	20	5/15	25	40/50	35	55/50	60	30/35	85	10/15	105	5/5	115
IX_{36}	10/10	20	20/0	25	0/5	35	40/40	60	35/20	85	15/15	105	5/5	115

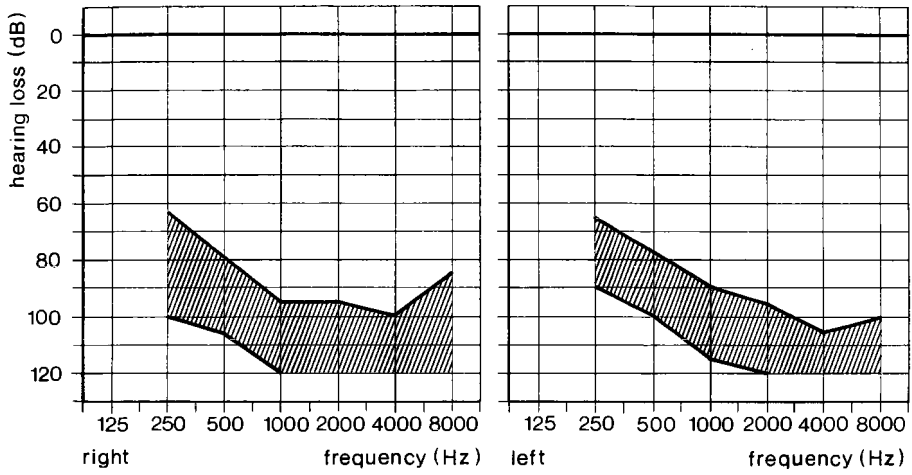

Figure 2: *Variability of pure-tone thresholds of this nonsyndromal type of autosomal recessive profound childhood deafness.*

Aulbers also failed to demonstrate abnormalities in the pure-tone audiogram that could indicate the carrier state in heterozygotes.[18,19,20] In the first three of these studies, it was likely that different autosomal recessive genes were involved in the deaf subjects who where investigated, and therefore also in their parents, each of which could lead to profound deafness in early childhood. In our study and those of Aulbers, however, it is almost certain that each of the two studies dealt with one and the same form of autosomal recessive disease.[19,20] As far as the nonsyndromal autosomal recessive profound childhood deafness that is described here is concerned, it is now clear that carriers of this autosomal recessive gene cannot be recognized, even using Békésy audiometry and stapedial reflex measurements. Since, on several occasions, it has not been possible to confirm the findings of Anderson and Wedenberg, and considering the doubts that the deafness in the children they studied could be attributed to the same autosomal recessive tendency, it becomes doubtful that their findings will ever be able to be reproduced.[13,14]

At present, it would appear that the problem of carrier detection can best be involved by using gene-linkage studies. Such studies would seem to be indispensable if the hereditary nature of nonsyndromal forms of profound deafness in the young child is ever going to be demonstrated with any certainty. At present, the failure to demonstrate any evidence for a noninherited cause for disease in the young child is considered, on empirical grounds, to indicate an increased risk (60% to 80%) of an autosomal recessive mechanism.[21]

Since it is likely that several genes are involved in causing nonsyndromal autosomal recessive profound childhood deafness, such an investigation into gene-linkage can only be adequately performed by examining large pedigrees that contain many individual family units.[10,11] Usually there are then several blood relationships. Such families arise especially in isolates that can occur on geographical, economic, or religious grounds. Western literature contains few publications on research into deafness in isolates.[22-27]. There are very few family studies such as the present report.[19,20,28-30]

It is only in the last few years that the availability of many new DNA markers has brought about greatly improved possibilities of locating specific genes by gene-linkage studies. At the same time, during the last few decades, the number of isolates in the western world has fallen greatly, as evidenced by the great reduction in the number of consanguineous marriages[31]; hence, there is the need for completing such gene-linkage studies in profound childhood deafness.

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Chapter Two

Branchio-Oto-Renal Syndrome

2.1 Shoulder Abnormalities in Association with Branchio-Oto-Renal dysplasia in a patient who has familial joint laxity*

Introduction

The association of earpits, branchial fistulae, deafness (sensorineural, conductive or mixed) and renal abnormalities, which constitute branchio-oto-renal dysplasia, is well known as an autosomal dominant condition, with an incidence of 1 in 40,000.[3] Cremers and Fikkers-van Noord found variable expressivity, and convincingly argue that other conditions such as the earpits-deafness syndrome and branchio-oto-renal dysplasia are, in fact, the same condition.[1] Associated abnormalities of pinna, face, lacrimal duct, palate, ureters and bladder have been described.[1,4,6] Associated shoulder abnormalities have not been described, but Fára et al described a man and 3 of his 7 children with oto-facial-cervical syndrome.[2] One other child, who died at 14 days, had preauricular fistulae. The described features were conductive hearing loss, prominent auricles with large conchae and preauricular and cervical fistulae. Sunken nasal root, narrow nose and long face were striking. They had long necks with sloping shoulders, prominence of the edge of trapezius, low set clavicles and laterally placed, winged scapulae. One child had agenesis of the right kidney. Their mild intellectual impairment was attributed to hearing loss.
No other cases have been described since, and McKusick suggests that it may be a variant of branchio-oto-renal dysplasia.[5]

Case-report

A 7 year old boy presented with an inability to elevate the left arm (actively or passively) beyond 110^0; all other shoulder movements were normal. He had a long neck and narrow, sloping shoulders, with a higher, laterally placed, winged left scapula (Figure 1). The free edge of trapezius was prominent. Radiographs showed no bony abnormality. In addition he had generalized joint laxity with normal skin (Ehlers-Danlos syndrome Type XI, McKusick 14790).[5] He was attending an ENT clinic where the diagnosis of branchio-oto-renal dysplasia had been made on the basis of moderate

*BH Pennie and HAM Marres MD. International Journal of Pediatric Otorhinolaryngology 1992; 23: 269-273.

Figure 1: *Sloping shoulders with the left higher and more laterally placed than the right (medial border of scapulae and spine marked).*

Figure 2: *Cup ear with ear pit (arrow).*

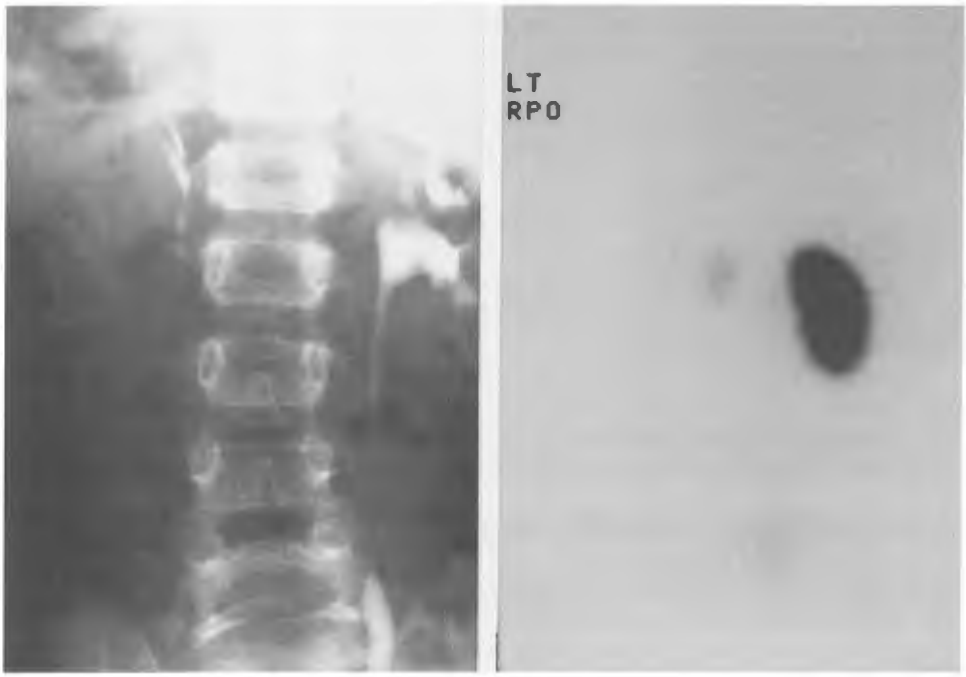

Figure 3: *Hypoplastic and dysfunctioning right kidney on intravenous pyelography (left) and renal scintigraphy (right).*

Figure 4: *Face of patient with a low nasal bridge and malformed ears.*

mixed deafness, preauricular pits, cup ears (Figure 2), branchial fistulae (excised at age 3 years) and a hypoplastic right kidney contributing 5% to total function (Figure 3). He had a low nasal bridge and rather a narrow face, but a normal palate (Figure 4). He is the second of three children of non-consanguinous parents both of whom have no siblings. His mother and two sisters also have generalized joint laxity with normal skin. His parents and siblings all have normal hearing on audiometry, normal kidneys on ultrasound, and none have any of the dysmorphic features discussed; a 4-generation family history was normal.

Discussion

This patient exhibits the major features of branchio-oto-renal dysplasia, and in addition most of the features described by Fára et al in their account of oto-facio-cervical syndrome; indeed his neck and shoulders bear a striking resemblance to their illustrations.[2]

We have not found the cause for limited shoulder movements in our patient, but it is likely to be a structural abnormality in the soft tissues of the shoulder girdle; the effects may have been mitigated by his coincident joint laxity. Since none of his relatives are affected, this patient appears to have a new mutation accounting for all the described features. Therefore we feel that it is extremely unlikely that these two conditions are separate entities. Shoulder abnormalities should therefore be evaluated in patients with branchio-oto-renal dysplasia.

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2.2 Neo-oval Window Technique and Myringo-chorda-vestibulopexy in the BOR-syndrome*

Abstract

Hearing improvement as a result of exploratory tympanotomy in BOR syndrome is disappointing. This is partly due to severe ossicular malformations and sometimes because of aplasia of the oval window as well. The neo-oval window technique is described in a patient with the BOR syndrome. In one ear a malleo-vestibulopexy was performed. In the other ear a myringo-chorda-vestibulopexy was the only option at time of surgery. Hearing improvement of 40 dB and 20 dB respectively was achieved.

Introduction

In about 10 to 15% of ears with a congenital minor ear anomaly, aplasia of the oval window is also present.[1] This figure can be expected to be even higher in the branchial arch syndromes. In the branchio-oto-renal (BOR) syndrome, referred to in the past as the ear pits deafness syndrome, aplasia of the oval window has been reported in a small series.[2] Compared to series with congenital minor ear anomalies including aplasia of the oval or round window, the results of exploratory tympanotomy in patients with this syndrome have been relatively disappointing as a result of the more complex ossicular chain malformations. Therefore the results of the neo-oval-window technique are reported in a patient with the BOR syndrome. The features of the BOR syndrome which were also present in the family members of this patient are also described.

Family study

A 15-year-old boy was referred to the Nijmegen Institute of ORL because of a bilateral 60 dB mostly conductive hearing loss which had been present since early childhood. Auricular appendages in the pre-auricular area had been removed. Both auricles

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*CWRJ Cremers, HAM Marres, HG Brunner. Laryngoscope 1993; 103: 1186-1189.

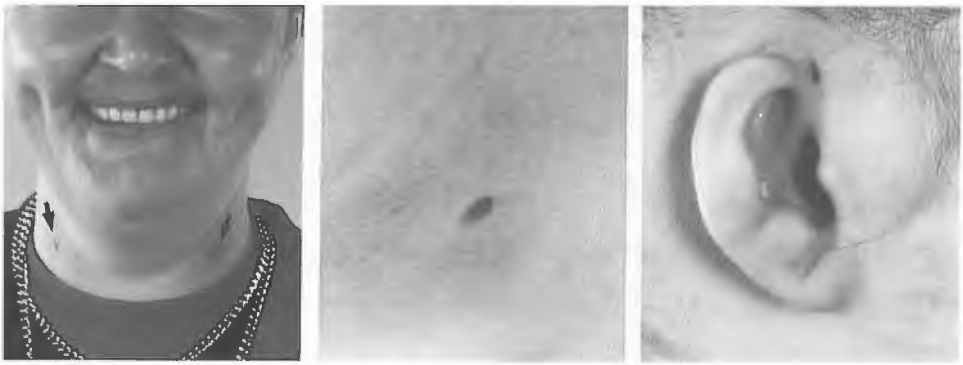

Figure 1: *Features of BOR-syndrome. Bilateral cervical fistulae in case II-4 (left) in detail of cercival fistula in case III-4 (middle). Pre-auricular sinus in combination with pinna dysplasia in case III-3 (right).*

showed underdevelopment of the superior helix. In 1977, reconstructive surgery of both auricles had been performed by cranial transposition of the superior pole of both cup ears and a free full-thickness skin graft from the retroauricular region.
On the left side of the neck a small fistula was present (Fig 1). There were no pre-auricular sinuses. No commissural lip pits were present either.
At otoscopy, the tympanic membranes were only partly visible as a result of curving of the external ear canal. Pure-tone audiometry showed a bilateral 60 to 70 dB mainly conductive hearing loss (Table I). Speech perception was 100% at 80 dB in the right ear.
Owing to the curving of the external acoustic canal, it was not possible to occlude the canal for stapedial reflex tests. CT scans of the petrous bones showed no pneumatisation of the mastoid and a low standing roof of the epitympanum. The vestibulum and the semicircular canals were of normal size.

Table I: *Pure tone-audiometry in 3 affected members of the family with the BOR syndrome. First number represents the bone conduction, second number represents the air conduction level (dB). When one number is given, only air conduction was measured (dB).*

Frequency kHz		0.25	0.5	1.0	2.0	4.0	8.0
II-4	R	35	20/35	10/25	35/35	5/20	35/80
	L	5	10	10	15	10	15
III-3	R	40	10/40	15/30	20/20	80/80	65/65
	L	60	25/70	20/80	30/70	15/60	10/65
III-4	R	60	5/55	5/55	15/55	10/60	5/55
	L	80	10/70	5/65	30/60	45/75	85

Table II: *Features of affected cases with the branchio-oto-renal syndrome. Case numbers refer to the pedigree in Figure 2.*

Patient	II-4		III-3		III-4	
Side	R	L	R	L	R	L
Pinna dysplasia	-	-	+	+	+	+
Pre-auricular sinus	+	+	+	+	-	-
Cervical fistula	+	+	+	-	+	+
Hearing loss	+	-	+	+	+	+
Renal abnormalities	-	-	-	-	-	-
Remarks	-		anotia and microtia		ear tags	

The diameter of the cochlea was considered normal as was the configuration of the internal acoustic canal. X-rays of the cervical, thoracic and lumbar spine showed no abnormalities. X-rays of the skull, hands, feet, arms and legs were also normal. A diagnosis of frontometaphyseal dysplasia was considered but rejected on the basis of the results of these radiological examinations. Renal function tests were normal as was the bilateral kidney ultrasound scan.

A limited family study was launched to try to confirm a syndromal diagnosis (Table II). The pedigree is shown in Figure 2.

The mother of the proband proved to have bilateral pre-auricular sinuses. The configuration of her ears was considered normal. Fistulae were present on both sides of her neck along the sternocleidomastoid muscle, which produced some clear secretions (Fig 1). There were no commissural lip pits and otoscopy was normal on both sides. Pure tone audiometry revealed a mild mixed hearing loss in her right ear, as presented in Table 1. Renal function tests and an ultrasound scan of her kidneys were normal. The grandfather of the propositus (I-1, not examined) was said to have had cervical fistulae and bilateral cup-ears. The only living sister (II-3) of the mother of the propositus and her two sons were not affected.

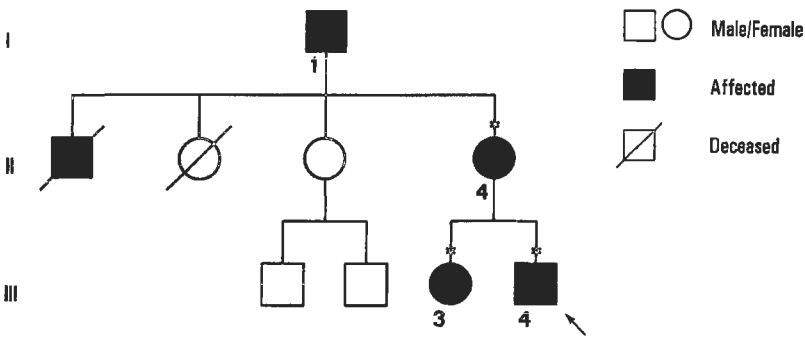

Figure 2: *Family pedigree with the BOR-syndrome. Marked (*) patients are examined, tested by pure tone audiometry and have had renal function tests and kidney ultra-sounds. Arrow indicates proband.*

The only sib of the propositus had left-sided anotia and microtia. The right auricle was smaller than normal, the superior helix was hypoplastic and there was a pre-auricular sinus (Fig 1). There were no commissural lip pits. A second branchial arch fistula on the right side of the neck had been excised. Otoscopy of the right ear showed a normal tympanic membrane and a well-aerated middle ear. The configuration of the malleus was normal. Increased hearing thresholds are shown in Table I. Renal function tests and ultrasound scans were normal.

Surgical technique

At the age of 16, exploratory tympanotomy of the right ear was performed in patient III-4. Before opening the middle ear, immobility of the malleus was demonstrated by manupulating the malleus with a probe. The long process of the incus, the suprastructure and stapes footplate were absent. At the site where the oval window niche would have been expected, there was a possible remnant of the annular rim. The round window niche was clearly well-developed. An aberrant and very thin chorda could not be preserved. The facial nerve was covered by bone.
To expose the epitympanum, mastoidectomy was performed but this proved to be so narrow that it was decided to continue surgery along the tympanotomy incision. Af-

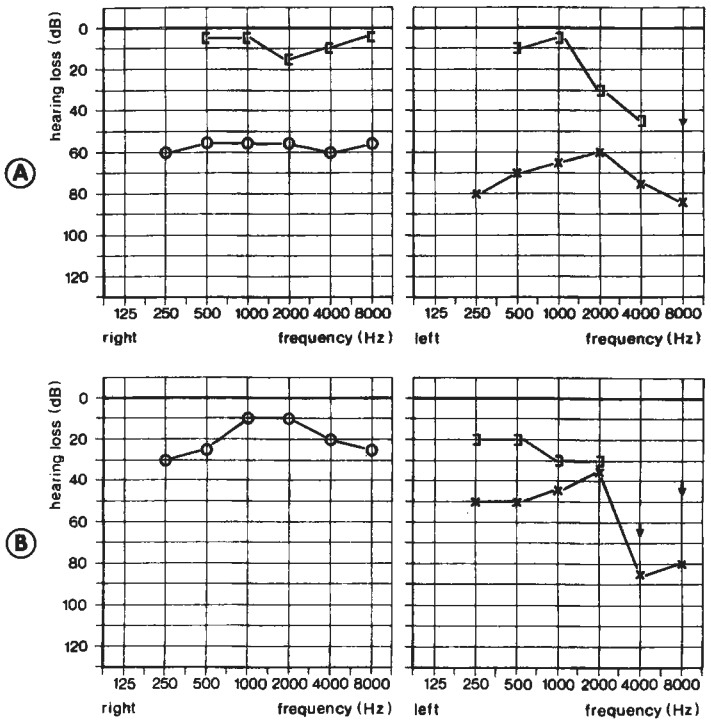

Figure 3: *Preoperative (A) and postoperative (B) hearing thresholds of proband (patient III-4) showing bone conduction hearing level ([,]) and air conduction hearing level (O , X).*

ter dislocating the incus posteriorly, the malleus became fully mobile. The head of the malleus was preserved. The periosteum was removed from the medial and posterior aspects of the malleus. A neo-oval window with a diameter of 0.6 mm was made at the site where the oval niche would have been expected. A teflon-platinum piston with a diameter of 0.4 mm and a length of 5.75 mm, measured from the middle of its opening, was placed around the neck of the malleus, projecting into the vestibulum. Hearing started to improve soon after surgery and only slight complaints of dizziness were noted during the first week postoperatively. There were no complaints of an altered sense or abcense of taste. The hearing level improved from 60 dB hearing loss to 10 dB during a follow-up of two years (Fig 3).

One year after the surgery on the right ear, the left ear was exposed under general anaesthesia. Testing of the mobility of the malleus, even before opening the middle ear, showed that it was fixed. The chorda was traced and preserved during surgery. The facial nerve was recognized and was covered by bone but the oval window niche could not been identified. The niche of the round window was clearly well-developed. There was no long process of the incus and again the stapes suprastructure and stapes footplate were absent. The mobility of the malleus was restored by dislocating the body of the incus posteriorly. A neo-oval window was drilled at a location somewhat anterior and lateral from the site where the oval window would be expected to

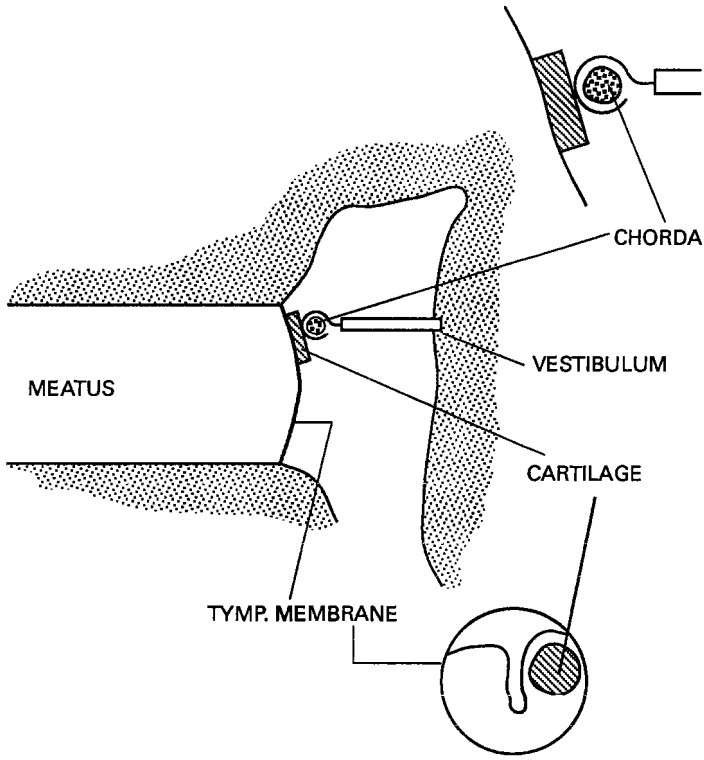

Figure 4: *Postoperative result (left ear); myringo-chorda-vestibulopexy with teflon-platinum piston (length 5.75 mm).*

originate. A 0.6 mm opening was created and after exposing the membranous structures, perilymph was found to be present. The distance between the malleus and the neo-oval window proved to be more than 6.5 mm, but a longer piston was not available at the time of surgery. Therefore it was decided to place a teflon-platinum piston around the chorda, with a diameter of 0.4 mm and a length of 5.75 mm, measured from the middle of its opening. The piston proved to be the correct length to project into the vestibulum and could be nicely crimped around the chorda. After this, autologous cartilage was interposed between the chorda and the tympanic membrane (Fig 4). There were no serious postoperative complaints and again no special sensations from the chorda were noted. The hearing level improved, as is shown in Figure 3 (follow-up one year). Although the hearing in the right ear is by far the best, the left ear is of some additional help and is used by the patient especially during phone calls.

Comment

An association between conductive or mixed deafness and cervical fistulae without pre-auricalar sinuses, is found to be present in about 11% of persons affected by the branchio-oto-renal syndrome.[3] It is therefore obvious that additional clinico-genetic evaluation of family members is necessary to diagnose the autosomal dominant inherited branchio-oto-renal (BOR) syndrome.

The unilateral aural atresia and microtia found in patient III-3 with the BOR syndrome, is a fairly unique symptom which has only been reported sporadically.[4]

In this study, great attention was paid to the existence of commissural lip pits, because commissural lip pits in combination with hearing loss, pinna dysplasia and preauricular sinus(es) can be an expression of a recently described autosomal dominant syndrome.[5] This syndrome should be distinguished from the BOR syndrome.

Reconstructive surgery of the middle ear in patients with the BOR syndrome has never been very successful.[2] There are no previous reports in the literature on the creation of a neo-oval window in patients with the BOR syndrome.

The creation of a neo-oval window is not generally accepted as a preferential technique for restoring hearing. Plester recommended a specific location for the neo-oval window when using this technique and demonstrated good results in ten consecutive cases.[6]

In his paper on congenital ear anomalies read at a Paediatric ORL meeting in Lódz, Poland in 1990, Helms reported that by following the recommendations made by Plester, treatment results could be matched, as was demonstrated in his own consecutive series of about ten cases. This induced us to perform a neo-oval-window technique on both ears of patient III-4. To our knowledge, myringo-chorda-vestibulopexy, as was performed on the left ear, is a new technique which has not been previously reported. The root idea came from Jahrsdoerfer from Houston, who told one of the authors that he had successfully interposed a teflon piston from the chorda into the vestibulum in a patient with congenital aural atresia and a missing long process of the incus.

The hearing in the left ear of patient III-4 would probably have been even more improved if a sufficiently long piston had been available during surgery which could have been interposed from the malleus into the vestibulum. In patients with congen-

ital middle ear anomalies, the malleus is often encountered in a more anterior position, which requires a long piston for malleo-vestibuloplexy.
Although the follow-up of this patient was fairly short, we are stimulated and pleased by the results of surgery and hope that this will open the way for hearing improvement in patients with congenital aplasia of the oval window in combination with ossicular chain anomalies.

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2.3 Autosomal dominant Branchio-Oto-Renal syndrome localization of a disease gene to chromosome 8q by linkage in a Dutch family*

Abstract

Branchio-Oto-Renal syndrome (BOR) is an autosomal dominant disorder with variable clinical manifestations affecting branchial, renal and auditory development. Varying clinical expression of the disease between different families suggests that multiple loci may be involved. However, the possibility of genetic heterogeneity as the cause of clinical variability cannot be resolved until the gene(s) causing BOR syndrome are mapped. DNA from four generations of a family with autosomal dominant BOR syndrome have been typed with a series of genetic markers on the long arm of chromosome 8. Using two point linkage analysis, a significant lod score of $Z = 4.0$ at $\theta = 0.05$ was obtained with the D8S165 microsatellite marker. Multipoint analyses with 8q markers place the gene for BOR between the markers D8S87 and D8S165.

Introduction

Branchio-Oto-Renal syndrome (BOR, MIM # 113650) is an autosomal dominant disorder characterized by ear malformations, cervical fistulae, hearing loss and renal abnormalities.[1] The prevalence of BOR syndrome is approximately 1:40,000, and it has been reported to occur in about 2% of profoundly deaf children.[2]
In 1864, Heusinger first described a condition in which preauricular pits, branchial fistulas and hearing impairment occurred together.[3] However, the first report of a family with associated kidney problems did not appear until 1967.[4]
Several families have been described since which exhibit both branchial cleft anomalies and preauricular pits inherited together in an autosomal dominant fashion.[5-6] Many families have been described with branchial cleft anomalies, preauricular pits and malformed auricles associated with deafness.[7-12]

*Shrawan Kumar, William J Kimberling,Judy B Kenyon, Richard JH Smith, Henri AM Marres, Cor WRJ Cremers. Human Molecular Genetics 1992; 1: 491-495.

Clinical studies suggest at least two syndromes. The first, Branchio-Oto-Renal (BOR) dysplasia, is associated with renal anomalies, but the second, Branchio-Oto (BO), lacks renal anomalies. In another family, renal involvement was limited to duplication of the collecting system and bifid renal pelves, which has been designated as Branchio-Oto-Uretral (BOU) syndrome.[13] Both BO and BOU phenotypes occurred in at least one large four generation kindred suggesting different manifestations of a single mutant gene.[14] Within a given family, the BOR mutation can express as either sensorineural, conductive or mixed hearing loss and does not always produce renal defects. The question arises as to whether BOR and BO are due to different alleles at the same locus or whether they are due to mutations at totally separate loci. The possible explanations for clinical heterogeneity are: 1) presence of different alleles at the same locus, 2) mutations affecting contiguous genes, 3) influence of the 'normal' allele on expression of the mutant BOR gene, 4) mutation at different locus, and 5) effect of the non-allelic modifiers (including environmental effects, genetic background, imprinting, etc.).
The complex symptomatic features of the BOR syndrome cannot be resolved until the gene causing the disease is localized and identified. Recently, the disease locus had shown a positive lod score for the PENK marker on chromosome 8q in another set of BOR families.[15] The clue to possible linkage to 8q was based on the report of an individual with manifestation of both tricho-rhino-phalangeal and BOR with an inherited rearrangement of the chromosome 8q region.[16]
The present study was undertaken to identify the site of the autosomal mutation responsible for BOR syndrome segregating in a large kindred from The Netherlands. We report the results of a series of genetic markers examined for genetic linkage which confirm and refine the assignment of the BOR locus in this family to chromosome 8q.

Material and Methods

Families and collection of blood samples
Blood samples were drawn from 24 informative individuals, of which 10 were affected, from a multi-generation family with branchio-oto-renal syndrome ascertained in The Netherlands. Lymphocyte transformation of the samples was performed, enabling permanent access to the samples for DNA extraction. The pedigree is shown in Figure 1. The details of the clinical evaluation and history have been described earlier.[23]

Polymorphic markers
Individuals were typed using microsatellite polymorphic markers from chromosome 8q. Details of the microsatellite markers are given in Table I (James Weber, personal communication). Oligonucleotide primers were synthesized using a Cruachem system synthesizer.

DNA extraction
Genomic DNA was extracted (500 µg to 2 mg) either from white blood cells or transformed lymphocytes by sodium dodecyl sulfate (SDS) lysis, proteinase K digestion,

Table I. *Description of the dinucleotide repeat polymorphic microsattelite markers used in localizing the BOR gene*

Locus Symbol	Cytogenetic Map Location	Oligonucleotides for PCR Amplification	Number of Alleles	Heterozygosity	PIC	Product Size Range in bp (Predominant Allele)
D8S87	8p21-Cen	5'-GGGTTGGTTGTAAATTAAAAC-3'	7	0.71	0.651	
(MFD # 39)		5'-TGTCAAATACTTAAGCACAG-3'				145–157(151)
D8S165	8q11-q13	5'ACAAGAGCACATTTAGTCAG-3'	7	0.54	0.50	
(MFD # 117)		5'-AGCTTCATTTTTCCCTCTAG-3'				138–152(142)
PENK	8q23-q24	5'-TAATAAAGGAGCCAGCTATG-3'	5	0.60	0.43	
(MFD # 31)		5'-ACATCTGATGTAAATGCAAGT-3'				75–83(79)
D8S166	8	5'-GATTGTGTCATTGCACTCCA-3'	10	0.88	0.83	
(MFD # 159)		5'-ACAAGGAAGTTCCTTTTTGG-3'				110–132(116)
D8S164	8q13-q22	5'-GATCATGTGAGTTAATACTTAAT-3'	14	0.86	0.79	
(MFD # 104)		5'-TCAGCTGCCTGTATTACTCA-3'				165–199(171)
D8S167	8q22-qter	5'-TTGTTCCTTTTCATGGCTGA-3'	14	0.84	0.86	
(MFD # 185)		5'-CAACTTATATATATTCCATGGC-3'				105–135(115,127)
D8S85	8q21-q22	5'-AGCTATCATCACCCTATAAAAT-3'	5	0.79	0.69	
(MFD # 18)		5'-AGTTTAACCATGTCTCTCCCG-3'				74–84(82)
D8S199	8q22-qter	5'-CCTTCTTTTTCTGCTCTGCT-3'	11	0.83	0.81	
(MFD # 177)		5'-AGTCACAGAGTAAATGATGG-3'				204–230(220)
\D8S198	8	5'-TAGGGACTACACATGATGGA-3'	10	0.83	0.81	
(MFD # 169)		5'-AACCAGATTAGGGACAAAGA-3'				155–173(161,163)

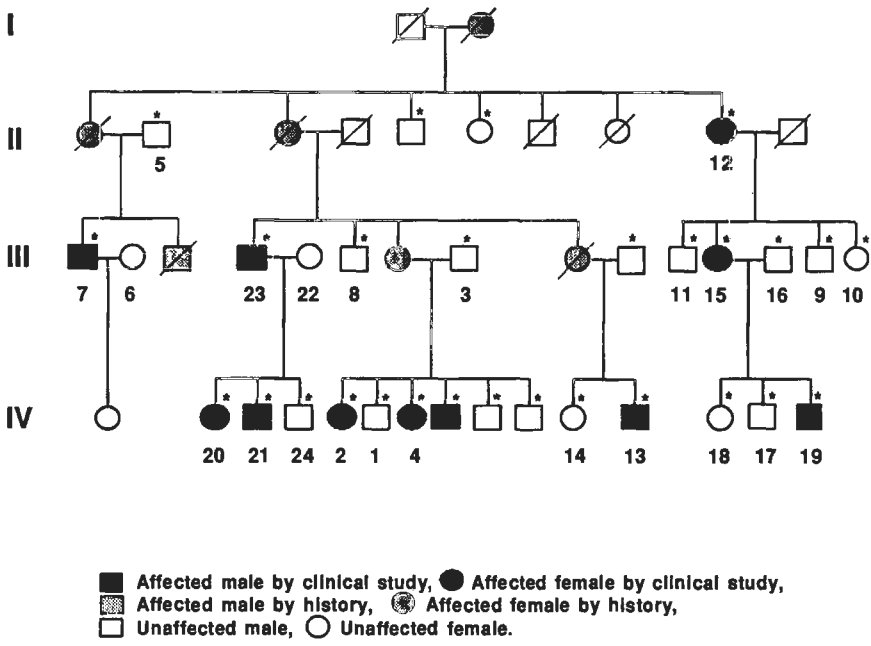

Figure 1: *Pedigree of BOR family. The individual numbers indicated in pedigree corresponds to the number shown in multiplex PCR results of marker D8S165 and PENK in figure 2. All the individuals shown in asterisk were clinically examined.*

phenol/chloroform extraction, and ethanol precipitation using an Applied Biosystems Nucleic Acid Extractor.

PCR amplification of genomic DNA

PCR amplification of genomic DNA from BOR family members was performed in an automated thermocycler (COY Inc.). Sample DNA reactions were carried in a volume of 25 to 100 µl. A standard reaction mixture contains the sample DNA (50 to 100 ng) to be amplified, two primers (20-50 picomole of each primer), Taq DNA polymerase (1-4 units for each sample), 200 µM dGTP, dCTP, dTTP, 2.5 mM dATP in a buffered solution (10 mM Tris-HC1-pH 8.8, 50 mM KC1, 1.5 mM $MgCl_2$, 0.1% Triton X 100) and 1µCi ^{32}P-dATP at 800 Ci/mmol for each sample. Samples were overlaid with mineral oil (Sigma) and the template DNA is first denatured by heating, followed by polymerase chain reaction.[24] Multiplex PCR typing, as shown in Figure 2, of PENK and D8S165 markers on chromosome 8 is carried out in the following way. The PCR amplification was performed in 25 µl volume for each sample and reactions containing 50 ng of genomic DNA template, 20 ng each of the four oligonucleotide primers, 1 µCi ^{32}P at 800 Ci/mmoles and 0.5 units of Taq DNA polymerase. DNA was amplified using synthetic oligonucleotide primers through 26 temperature cycles consisting of 1 min at 94^{0}C (denaturation), 2 min at 55^{0}C (annealing), and 2 min at 72^{0}C (extension), and the last extension was lengthened to 5 minutes.

Five microliters of polymerase chain reaction product of each sample were mixed with ten microliters of gel loading dye buffer containing formamide (98% deionized for-

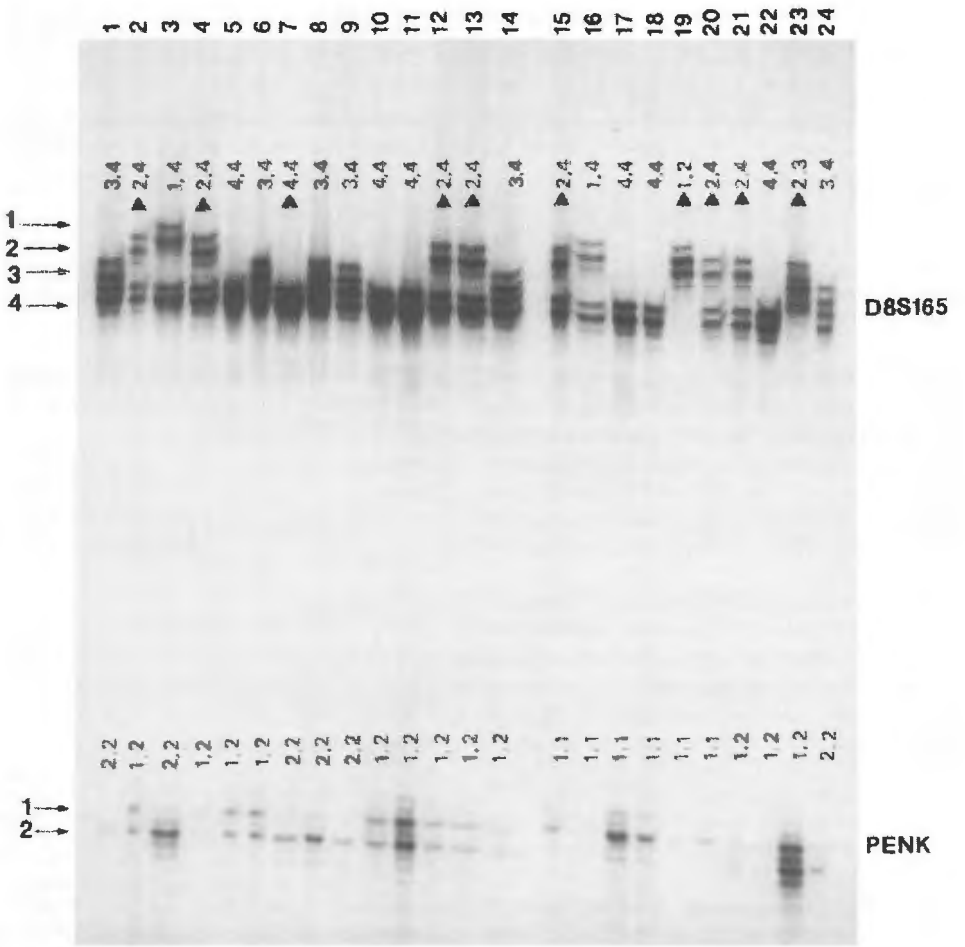

Figure 2: *Multiplex PCR DNA typing on a sequencing gel. The genotypes for different persons are indicated in each lane. The numbers on the top in each lane corresponds to the individual number shown in pedigree (figure 1). The genotypes of an affected individuals are indicated by a triangle.*

mamide, 10 mM EDTA (pH 8.0), 0.025% xylene cyanol FF, 0.025% bromophenol blue), and samples were denatured by boiling for 3 minutes before loading. In each lane four to five microlitres of denatured sample was loaded and electrophoresis was carried out in 6% denaturing polyacrylamide DNA sequencing gel (Fisher Biotech, FB-Seq-3545), containing urea, in 1 x TBE buffer at 70 watts. The urea was removed after electrophoresis in 5% Acetic Acid and 15% Methanol, gel was dried and autoradiographed overnight on Kodak XAR-5 film.

Data analysis

Linkage analysis was performed on a personal computer using LINKAGE program version 5.1.[25-26] Pairwise lod scores were calculated using MLINK and ILINK options of LINKAGE package. The LINKMAP subprogram of the computer package LINKAGE

(Version 5.1) was used in the multipoint analysis. Multipoint lod scores were calculated as $\log_{10}$ [L(x)L(∞)], where x is the distance of the BOR locus relative to a fixed point on the established map of loci and where (∞) represents an infinite map distance corresponding to θ=0.05. The recombination difference for θ_m/θ_f was assumed to be 2.20 from male and female genetic map of chromosome 8 for the multipoint analyses (James Weber, Helen Donis-Keller, personal communication). The recombination fractions used and the order of chromosome 8 loci was assumed to be:

D8S87 D8S165 PENK D8S166 D8S164 D8S167 D8S85 D8S199 D8S198
cen|----0.21----|----0.001----|----0.04----|----0.32----|----0.15----|----0.25----|----0.06----|----0.02----| qter

Only genotypes that were distinct in PCR results for each individual were included in the linkage analysis, and dominant mode of inheritance with full penetrance was assumed. No inferred genotypes were included in the statistical analysis.

Results and Discussion

The two-point linkage analysis results are presented in Table II. A lod score of -2.0 significantly excludes the disease locus from the marker at the recombination fraction indicated whereas a lod score of 3 and above is an indication in favor of linkage (odds 1000:1 in favor of linkage). Based on the pairwise lod score analyzed for each marker, a maximum lod score of 4.0 at θ= 0.05 was obtained for the marker D8S165 with BOR (Table II). Other markers such as PENK, D8S166, D8S167 also yielded positive, but not significant, lod scores with BOR in this family. The PCR typing results of D8S165 and PENK are presented in Figure 2. It is clearly evident from the results of ten affected individuals, indicated by a triangle in Figure 2, that the disease gene is segregating with allele 2 for marker D8S165. Only one recombination event is observed (individual number seven).
This study localizes the disease locus in this family to chromosome 8q by virtue of the LOD score at 4.0 at a recombination fraction of 5% between D8S165 and BOR. Based on multipoint results, the disease locus is placed between the markers D8S87 and

Table II. *Two point lod score of different polymorphic markers on chromosome 8 with BOR*

	*Recombination factor θ**						
Markers	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D8S87	−∞	−2.34	−0.91	−0.34	0.08	0.17	0.12
D8S165	2.16	3.67	4.0	3.82	3.10	2.14	1.03
PENK	−1.08	0.48	1.08	1.21	1.06	0.69	0.26
D8S166	−0.289	1.25	1.75	1.81	1.53	1.06	0.47
D8S164	−∞	−2.48	−1.12	−0.57	−0.14	−0.01	0.01
D8S167	−∞	−1.61	0.13	0.64	0.75	0.55	0.26
D8S85	−∞	−3.43	−1.23	−0.43	0.19	0.32	0.21
D8S199	−∞	−4.41	−1.79	−0.79	−0.01	0.21	0.19
D8S198	−∞	−2.30	−0.26	0.45	0.81	0.70	0.38

* Lod scores were calculated assuming θ *male* = θ *female*

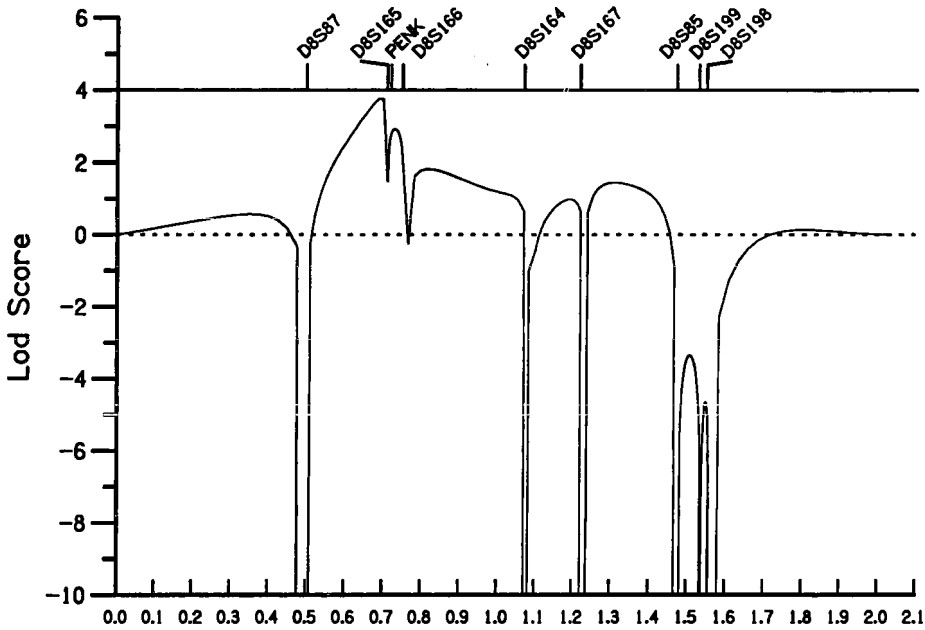

Figure 3: *Multipoint analysis with BOR and the markers on chromosome 8q place the disease locus between the markers D8S87 and D8S165. Weber's 8q map results (J. Weber, personal communication) were used to account for the differences between genetic distances and recombination fractions of the chromosome 8q markers.*

D8S165 (Figure 3). The multipoint analysis of the PENK with other markers place the PENK gene closer to D8S165 (results not presented). The results support the previous finding of a suggestive positive lod score with PENK marker (Z=2,48, θ=0.065) in another BOR family.[15] A multipoint lod score of 3.70 at θ_m=0.036 on the centromeric side of PENK was observed in that family. Since PENK and D8S165 markers are very close to each other, the positive lod scores with these markers in two different set of BOR families suggest that the disease is caused by the same mutation. Further mapping of these markers relative to the existing map could assist in producing a more refined map with sufficient information to allow us to flank the disease locus at a much closer distance.

It is evident from other studies that the phenotypic expression of the branchial arch, audiologic and renal development can be quite variable even within the same family.[14] As stated before, the nature of the intra and interfamilial variability of expression is unknown. The fact that many clinical features cluster in families suggests the possibility that there is a gene at another locus segregating in some families which produces a distinct genetic entity for a different syndrome.

There are also reports of overlapping features of hemifacial microsomia (HFM) and the branchio-oto-renal syndrome in which both syndromes present with similar features such as malformed auricles, preauricular appendages and/or pits, hearing loss, branchial cleft cartilage and facial paresis.[17-20] Several anomalies common to both the branchio-oculo-facial (BOF) syndrome and BOR have also been reported.[21] More re-

cently another autosomal dominant syndrome, which consists of deafness, preauricular sinus, external ear anomaly and commissural lip pits, but no cervical fistulae and renal anomalies, has been reported in a large pedigree.[22]

Now that the gene causing BOR syndrome has been localized, families with variable clinical manifestations (BO, BOU, HFM, BOF) can be investigated with chromosome 8q markers to resolve the issues of genetic heterogeneity. We are now in the process of collecting those families. If the variety of phenotypes (BO, BOU, HFM, BOF, BOR) are allelic, the spectrum of defects can be defined and will provide information to improve the clinical criteria for diagnosis.

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Chapter Three

A New Branchial Arch Syndrome

3.1 Congenital Conductive or Mixed Deafness, Preauricular sinus, External Ear Anomaly and Commissural Lip Pits: An autosomal dominant inherited syndrome*

Abstract

Branchiogenic syndromes such as branchio-oto-renal syndrome, Treacher Collins syndrome and hemifacial-microsomia are well delineated. From a clinical study in a large family spanning three generations, it can be concluded that the association of conductive deafness, commissural lip pits, preauricular sinuses, and external ear anomalies can be differentiated from the above mentioned syndromes and is a separate autosomal dominant syndrome.

Introduction

Congenital deafness is diagnosed in 1 in 1,000 children.[1] Inherited types of deafness are believed to be the cause in almost one half of these - thus, in 1 in 2,000.[2] In some of the inherited disorders, hearing loss is the only feature, whereas in others it may be associated with other abnormalities.[3,4] At present, more than 40 autosomal dominant syndromes with congenital hearing loss have been recognized.
An atypical presentation of the branchio-oto-renal (BOR) syndrome (also called ear pit-deafness syndrome) was thought to be the cause of impaired hearing in a father and his twin sons who were referred to the Nijmegen Otorhinolaryngology department for hearing evaluation and genetic counseling. The family history revealed additional affected family members, and in consequence, a detailed family study was launched.

Methods

A genealogical study was performed to complete a pedigree spanning three generations. All 74 living members, plus the spouses of persons II-1 and II-10, were asked to

*Henri AM Marres, MD, Cor WRJ Cremers, MD. Annals of Otology, Rhinology and Laryngology 1991; 100: 928-932.

participate in this study. Each person underwent general medical and detailed otorhinolaryngologic assessments. In all persons, any features that were present were recorded by photography.
All persons over the age of 3.5 years were tested audiologically by pure tone audiometry. If this consistently showed conductive or mixed hearing loss, impedance audiometry was also performed.
In four persons (II-10, proband III-7, IV-7 and IV-8) who were suspected of having an atypical presentation of the BOR syndrome, thorough renal assesment had been carried out previously, consisting of blood and urine chemistry, renal ultra-sound and intravenous pyelography. All of these findings were normal. Therefore, we decided to screen the other family members for signs of renal dysfunction using only biochemical methods. However, after 24 of the members were found to be normal, this screening was discontinued.

Results

The family pedigree is presented in Figure 1. All 74 family members, plus the spouses of II-1 and II-10, were examined by the authors. The presence of a pre-auricular sinus, commissural lip pits, an external ear anomaly and hearing impairment in the different family members is also shown in the pedigree.
An external ear anomaly, described as lumping and flattening of the superior part of the helix in combination with a reduction in the triangular fossa either unilaterally or bilaterally, was found in 12 persons. Its variability was only minor, as illustrated in Figure 2.
Eleven persons were found to have a unilateral or bilateral preauricular sinus (Figure 1). A preauricular cyst was palpable in case II-1 (left) and II-10 (right), which was also derived from the same branchial pouch, whereas there was no evidence of a pre-auricular sinus or ear pit.
In addition, we found one or more commissural lip pits in eight persons, which had not been noticed previous to the study (Figure 3). There were small sinuses, maximum 4 mm in depth, in the corner of the mouth. Although they can be saliva-productive, they are without clinical importance.
In conclusion, a total of 20 out of the 74 persons were affected by external ear anomalies, preauricular sinuses (or cysts) and commissural lip pits, either in combination or separately, as summarized in Table I.
General medical examination revealed mild enlargement of the thyroid gland in six female persons (II-10, II-11, III-27, III-29, III-32 and IV-34). A thyroid scan, done in all but one subject (II-11) of the six confirmed the existence of goiter in only two cases: II-10 and III-32. An Iodine uptake and perchlorate study performed in II-10, III-27, III-29, III-32 and IV-34 yielded normal results, so an iodination defect could be excluded.
To differentiate between various branchiogenic syndromes, it is important to mention that we were unable to demonstrate a cervical cyst or cervical branchial arch fistula in any of the 74 family members.

The hearing of 68 persons (spouses II-2 and II-9 inclusive) was assessed by means of pure tone audiometry. All the persons in the remaining group were younger than 3.5

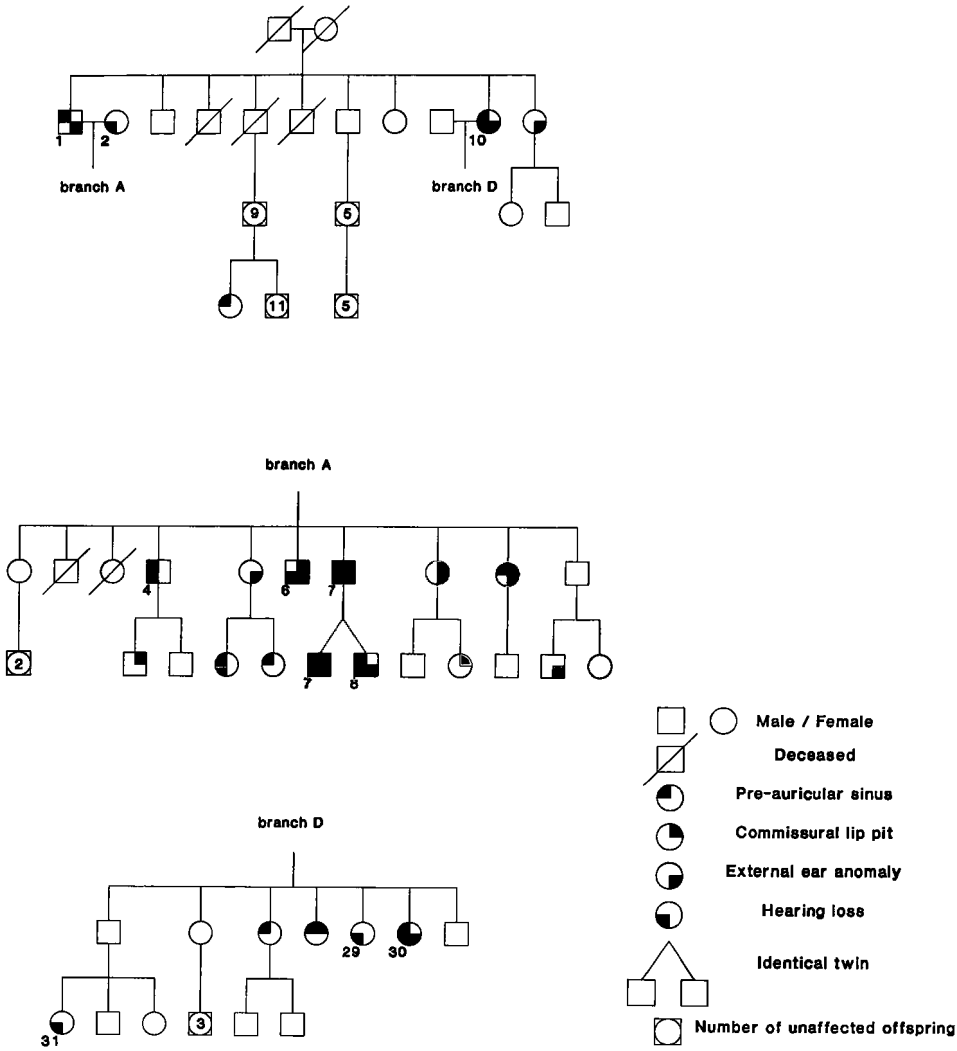

Figure 1: *Family pedigree. All living members underwent clinical examination. Results of clinical examination and audiologic tests are presented per individual.*

years of age and none of these children were affected by one of the aforementioned anomalies.

Elevated hearing thresholds (defined as a mean threshold > 25 dB at frequencies 500, 1,000 and 2,000 Hz) were found in 11 persons (Figure 1 and Table II). A combination of hearing loss with one of the phenotypic characteristics could be demonstrated in 8 persons. In 6 of them, the hearing loss could only be explained by a hereditary cause (II-10, III-4, III-7, III-30, IV-7 and IV-8). The elevated threshold was mainly conductive, especially in the lower and middle frequencies. Tympanometry, stapedial reflexes, and the operative findings in 3 of them confirmed a presumed middle ear, or more specific, congenital ossicular anomaly, such as stapes ankylosis, and the absence of

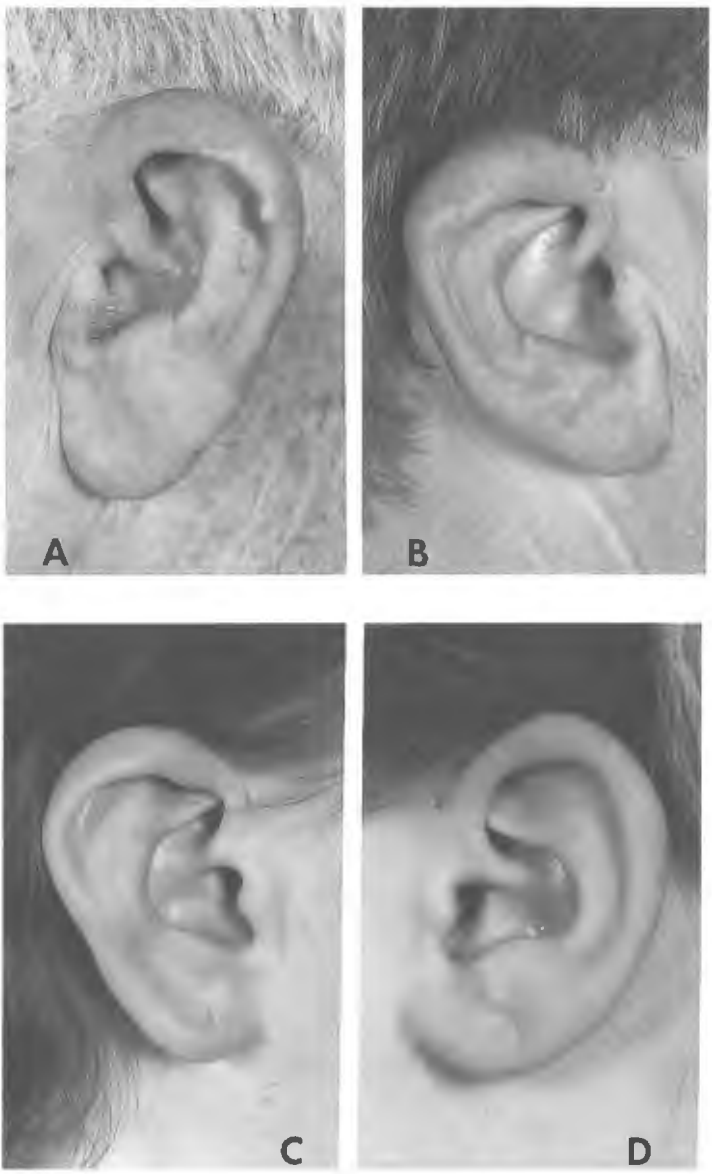

Figure 2: *Auricles of A) II-1, B) III-7, C) III-30, and D) IV-6. Three show anomaly of pinna. Preauricular sinus is not associated with external ear anomaly in IV-6.*

Figure 3: *Commissural lip pit in III-28.*

Table I: *Clinical features of 20 affected persons in three generations. Judgement of the ear anomalies may be biased in persons II-11, III-5 (Right) and III-30. + feature present, - absent, +/- biased.*

	Preauricular sinus		**Commissural lip pit**		**External ear anomaly**		**Congenital hearing loss**	
Code	Left	Right	Left	Right	Left	Right	Left	Right
II-1	Cyst	-	-	-	+	+	-	-
II-10	-	Cyst	-	-	+	+	-	+
II-11	-	-	-	-	+/-	+/-	-	-
III-4	+	-	-	-	-	-	-	+
III-5	-	-	-	-	+	+/-	-	-
III-6	-	-	-	+	-	+	+	-
III-7	+	+	+	+	+	+	+	+
III-8	-	-	-	+	-	+	-	-
III-9	+	+	-	+	+	+	-	-
III-27	+	-	-	-	-	-	-	-
III-28	+	+	+	+	-	-	-	-
III-30	-	+	-	-	-	+/-	+	-
IV-3	-	-	+	+	-	-	-	-
IV-5	-	+	-	-	-	-	+	-
IV-6	+	-	-	-	-	-	-	-
IV-7	+	+	+	+	+	+	+	+
IV-8	+	+	-	-	+	+	+	+
IV-10	-	-	-	+	-	-	-	-
IV-12	-	-	-	-	+	+	-	-
IV-16	+	-	-	-	-	-	-	-

Table II: *Pure-tone thresholds of 11 persons with hearing impairment. In pairs of numbers joined by plus symbool, first number is sensorineural component and second number is conductive component. Group A - six persons with hereditary deafness and phenotypic features as described in Table 1, group B - two persons with possibly acquired eafness and features, group C - two persons with acquired deafness without features and in-law II-2.*

			Frequencies (Hz)					
Group	Code	Ear	250	500	1,000	2,000	4,000	8,000
A	II-10	L	20	10+10	0+10	0+10	0+25	30+15
		R	65	10+60	10+60	45+30	30+45	80
	III-4	L	15	10	5	5	15	30
		R	50	5+35	5+35	10+25	0+40	5+40
	III-7	L	40	0+45	5+40	5+30	5+40	0+45
		R	50	0+45	5+40	10+45	10+40	5+40
	III-30	L	50	15+25	10+40	20+25	25+40	75
		R	10	10	10	20	35	50
	IV-7	L	-	60	55	50	45	65
		R	-	10+45	5+55	10+25	0+20	10+20
	IV-8	L	-	40	45	25	25	-
		R	-	0+55	0+45	5+30	0+35	-
B	III-6	L	60	10+40	5+20	10+10	5+35	30+15
		R	30	5+15	5+5	10+0	15+40	40+5
	IV-5	L	60	0+35	0+30	0+25	5+25	40+5
		R	15	5+10	5+15	0+5	5+0	25+15
C	II-2	L	55	50	40	60	75	70
		R	85	85	70	45	55	80
	III-29	L	40	5+45	15+40	25+30	25+35	35+25
		R	55	0+50	0+45	15+30	10+20	15+20
	IV-31	L	-	0+35	0+40	0+30	0+25	-
		R	-	20	30	20	20	-

the long process of the incus. Stapes interpositioning was performed in two ears (proband III-7). In one ear (III-30), reconstruction of the ossicular chain with an allograft was carried out. An exploratory tympanotomy was carried out in one ear (II-10) and revealed aplasia of the round window.

Mild progression of the hearing loss could be demonstrated when previous audiograms (performed 1 to 21 years previously) were compared to the present audiograms. The medical history and otologic examination of the two remaining hearing-impaired persons with phenotypic features (III-6 and IV-5) indicated a hereditary cause for their hearing loss as well, but an acquired cause could not be excluded. Exploratory tympanotomy has not been proposed.

In the two cases in hearing loss without any of the phenotypic features (III-29 and IV-31) and also in II-2, the medical history and otorhinolaryngologic examination confirmed the diagnosis of acquired hearing loss. It is important to note that the progressive mixed deafness in case II-2, who is the mother of the proband and the spouse of family member II-1, is very suggestive for otosclerosis. However, the hearing loss of her offspring (III-4, III-6 and III-7) is congenital and only mildly progressive, and stapes ankylosis has been demonstrated.

Discussion

Preauricular sinuses, pinna dysplasia and deafness can be expressions of branchiogenic disorders as observed in Treacher-Collins syndrome, the BOR-syndrome, and the hemifacial microsomia syndrome.[5-7] But these features can exist as seperate entities as well.

A preauricular sinus or ear pit is found in 1% of the white population, with an autosomal dominant mode of inheritance.[4,8] Commissural lip pits, contrary to medial lip pits, are said to be even more common; percentages reaching a maximum of 12% have been described.[9,10] Baker[9] demonstrated that there is a significant correlation between the incidence of preauricular sinuses and commissural lip pits: of all the persons with a commissural lip pit, 3.8% also proved to have a preauricular sinus. In our study, a preauricular sinus was found in four of the eight persons with a commissural lip pit. Congenital pinna dysplasia and middle ear malformations are sequellae of disrupted genesis from the six hillocks of the first two branchial arches and the cartilage of Meckel and Reicher, respectively.[11-13] The judgment and interpretation of abnormalities is difficult and often subjective. Nevertheless, in this family, it was thought that the external ear malformation was quite specific.

The association between congenital conductive or mixed hearing loss and preauricular sinuses, commissural lip pits and external ear anomalies, was so remarkable in this pedigree that it leads us to the conclusion that genetic disease is present. The pattern of inheritance has to be autosomal dominant, because this syndrome proved to be present in three generations and there were male-to-male transmissions as well.

Although several of these features also form part of the BOR-syndrome, which is well known to the present authors, we consider that the syndrome reported here is a different entity, mainly because of the absence of cervical fistulae and renal abnormalities and the presence of commissural lip pits.[5,14]

We therefore conclude that the symptoms described in this study represent a congenital syndrome with an autosomal dominant pattern of inheritance and variable penetrance and expression of the features, that has not been reported previously.

In the future, gene-linkage studies are expected to be useful for making a definite differentiation between the various branchiogenic autosomal dominant inherited syndromes.

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3.2 The deafness, pre-auricular sinus, external ear anomaly and commissural lip pits syndrome. Otological, vestibular and radiological findings*

Abstract

Commissural lip pits, pinna dysplasia, pre-auricular sinus and hearing loss constitute a recently described autosomal dominant branchial arch syndrome. In a large family, 8 out of the 74 members were also affected by conductive hearing loss. No inner ear abnormalities could be demonstrated on the CT scans. In 3 patients (4 ears) out of 4 patients (6 ears), exploratory tympanotomy revealed serious ossicular chain anomalies. In one ear, round window aplasia was also present.
Long-term hearing improvement could only be achieved in one ear.

Introduction

Various autosomal dominant hereditary branchial arch syndromes with symptoms including pinna dysplasia, pre-auricular sinus and congenital conductive or mixed hearing loss have been described. The best known examples are the Branchio-Oto-Renal (BOR) syndrome and the Treacher Collins syndrome or mandibulofacial dysostosis.[1] Gene-linkage has recently been successful for these two syndromes. The BOR syndrome has been linked to chromosome 8q and the Treacher Collins syndrome to chromosome 5q.[2,3]
The results of clinical examination of the family described in this paper indicate that this is a new autosomal dominant inherited syndrome, whose symptoms comprise pre-auricular sinus, commissural lip pits, external ear anomaly and also mixed or conductive hearing loss (MIM # 120502).[1,4] At present, gene-linkage studies are being performed on this family to investigate whether the distinction between this syndrome and the above-mentioned branchial arch syndromes can be supported by the results of gene-linkage studies.

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The otological aspects of this syndrome are described in more detail, together with the findings and results of middle ear surgery.

Patients and methods

The proband was examined at the Nijmegen Department of Otorhinolaryngology for the evaluation of congenital hearing loss.
The request for genetic counseling subsequently arose. This led to further differentiation and analysis of the features in the proband and several members of his family. Although it could be concluded that the hearing loss, pinna dysplasia and pre-auricular sinus displayed an autosomal dominant inheritance pattern, it was difficult to distinguish these symptoms from the branchio-oto-renal (BOR) syndrome (Table I).
We were recently offered the opportunity to perform a detailed family study on the 74 family members.
All the subjects underwent oto-rhino-laryngological examination, including microotoscopy and all the persons older than 3.5 years (n=66) also underwent pure tone audiometry. The findings of this examination are shown in the pedigree (Figure 1).
The study results led us to the conclusion that this might be a new autosomal dominant branchial arch syndrome.[4]
The features of this syndrome were: conductive or mixed hearing loss, preauricular sinus or cyst, external ear anomalies and commissural lip pits (Figure 2).
Contrary to the BOR-syndrome, there were no renal anomalies and cervical fistula.
One or more features were found in 20 persons which could be attributed to this syndrome.
Within the latter group, 8 persons were also suffering from hearing loss (Tables II and III). Their data formed the subject of this study. Ear surgery was performed on 4 persons (6 ears), 6 underwent high resolution CT scanning of the middle and inner ear.

Table I: *Three branchial arch syndromes. The penetration and variation of the features are not presented in the table (+ feature compatible with the syndrome, - feature not described in association with the syndrome).*[4,7,17]

Features	The Treacher Collins syndrome	The BOR syndrome	The present syndrome in this pedigree
Pinna dysplasia	+	+	+
Preauricular sinus	+	+	+
Ear appendage	+	+	-
Aural atresia	+	+	-
High arched or cleft palate	+	+	-
Hearing loss	+	+	+
Cervical fistula	-	+	-
Renal anomalies	-	+	-
Hypoplasia or aplasia of facial bones	+	-	-
Commissural lip pits	-	-	+
Vestibular hypofunction	-	+	-

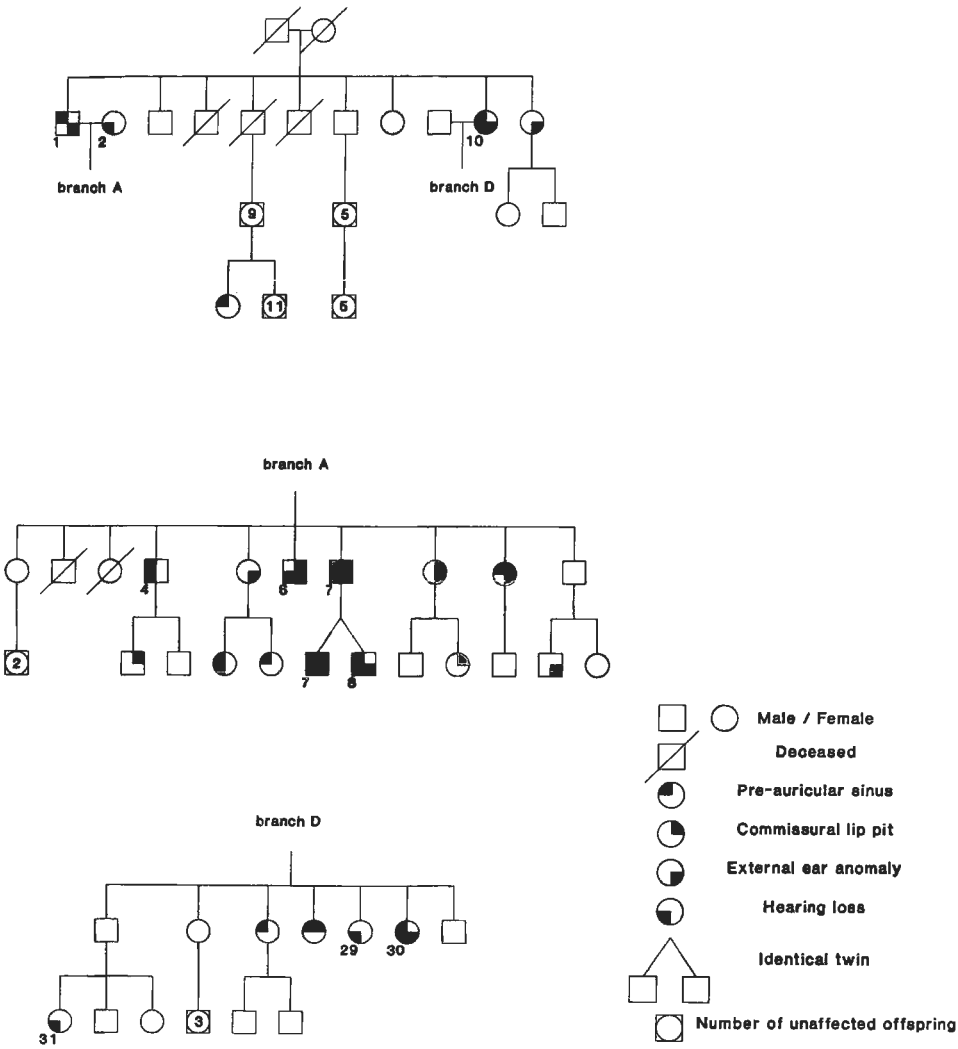

Figure 1: *Pedigree of a four-generation family. All the family members in three generations were examined (n=74). All persons older than 3.5 years were tested audiologically (n=66).*

Vestibular examination with electronystagmography and computer analysis was performed in three cases. The tests included velocity step tests and caloric tests, as well as smooth pursuit and cylindrical-screen optokinetic nystagmus tests. Furthermore it was assessed whether any gaze nystagmus (on visual fixation in lateral gaze) or spontaneous nystagmus (eyes open in the dark) occurred.

Results

Patient 1: (III-7 in Figure 1): In 1962, the proband aged 8 years, was referred to the Department of Otorhinolaryngology because of suspected bilateral congenital hear-

Figure 2: *A. Pre-auricular sinus with (middle) and without (left) pinna dysplasia. Pinna dysplasia without pre-auricular sinus (right). B. Commissural lip pit (arrow).*

ing loss. The medical history did not reveal any etiological factors.
Physical examination showed bilateral pinna dysplasia and a pre-auricular sinus (Table II). Recently as part of a family study, commissural lip pits were also found to be present [Marres 1991]. Hearing thresholds were obtained by regular pure-tone audiometry from 1962 to 1991 (Table III). A hearing aid was fitted at our subunit for paediatric audiology. He received personal tuition to help with his schooling.
To improve his conductive hearing loss, exploratory tympanotomy was performed on his right ear at the age of 12 years. The incus and malleus were found to be mobile but there was a plump ankylotic stapes. The long process of the incus was fragile and

Table II: *Patient characteristics. Numbers between brackets refer to the pedigree in Figure 1.*

Patient	Pre-auricular sinus		Commissural lip pit		external ear anomaly	
Ear	right	left	right	left	right	left
1 (III-7)	+	+	+	+	+	-
2 (III-30)	+	-	-	-	+	-
3 (II-10)	cyst	-	-	-	+	+
4 (IV-5)	+	-	-	-	-	-
5 (III-6)	-	-	+	-	+	-
6 (III-4)	-	+	-	-	-	-
7 (IV-7)	+	+	+	+	+	+
8 (IV-8)	+	+	-	-	+	+

too short. There was no bony contact between the incus and the stapes. The round and oval windows were found to be normal, the facial nerve had a normal course and the bony canal was intact. Stapedectomy was performed and a 3.5 mm teflon wire piston was interposed on to the incus. The hearing improved post-operatively from about 50 to 20 dB hearing threshold. The patient no longer found it necessary to wear a hearing aid (Table IV).

Table III: *Elevated hearing thresholds of 8 patients with associated anomalies as shown in Table II. The first number represents the air conduction threshold (in dB HL), the second number (if measured) is the sensorineural hearing threshold. (if the patient underwent ear surgery, the preoperative thresholds are presented).*

Patient	Ear	Frequency (Hz)					
		250	500	1000	2000	4000	8000
1	left	40	45/0	45/5	35/5	40/5	45/0
	right	50	45/0	45/5	55/10	50/10	45/5
2	left	50	40/15	50/10	45/20	65/25	75
	right	10	10	10	20	35	50
3	left	20	20/10	10/0	10/0	25/0	45/30
	right	65	70/10	70/10	75/45	75/30	80
4	left	60	35/0	30/0	25/0	30/5	45/40
	right	50	40/0	25/0	20/0	20/0	50/25
5	left	60	50/10	25/5	20/10	40/55	45/30
	right	30	20/5	10/5	10/10	15/55	45/40
6	left	15	10	5	5	15	30
	right	50	40/5	40/5	35/10	40/0	45/5
7	left	-	60	55/0	50/10	45/5	65
	right	-	55/10	60/5	35/10	20/0	30/10
8	left	50	45/5	40/5	25/10	30/15	45
	right	50	50/0	45/0	40/5	35/0	45

Table IV: *Post-operative hearing thresholds. The first number represents the air conduction threshold (in dB HL), the second number (if measured) is the sensorineural hearing threshold.*

			Frequency (Hz)					
Patient	Ear	Follow-up (yrs)	250	500	1000	2000	4000	8000
1	right	22	25	20/0	20/5	10/10	35/20	45/30
	left	1	40	50/5	35/20	20/15	30/5	70/20
	left	0.5	40	45/5	45/5	30/15	60/15	55/25
	left	4.5	50	45/0	45/5	55/10	80/30	120
	left	2	60	45/10	40/5	25/20	70/35	80
2	left	7	50	40/15	50/10	45/20	60/45	75
	left	1	50	45/10	45/10	30/20	45/30	80
3	right	1	60	65/25	70/10	85/45	75/40	80
4	right	1	15	15/5	20/5	5/0	5/5	40/25
	left	2	65	50/5	25/5	25/5	45/15	65

Exploratory tympanotomy on the left side was performed at the age of 26 years. Stapes reflexes were not measured pre-operatively. During surgery, the malleus was found to be hypermobile, the long process of the incus was almost absent and the stapes was malformed and fixed through ankylosis to a normal sized oval window. The facial nerve showed no abnormalities in its course and had an intact bony canal. A teflon wire piston with a length of 5 mm was interposed between the malleus and vestibule after stapedotomy. His post-operative hearing remained unsatisfactory, so the ear was re-explored to improve the teflon interpositioning. This achieved a mean hearing gain of 25 dB. However, during follow-up, the hearing thresholds increased (Table IV) and the same procedure was repeated. Again, piston interpositioning was improved in combination with re-opening of the oval window. The teflon wire piston was attached around the neck of the malleus without removing the head of the malleus. Only the neck of the malleus and part of the handle were detached from the tympanic membrane. However, in the follow-up period, the wire of the piston penetrated the tympanic membrane and was extruded. The same surgical technique was subsequently repeated in combination with a fascia underlay technique to cover and support the piston-malleus connection. These repeated operations on the left ear were mainly conducted at the request of the patient, who had experienced what it meant to be able to hear with both ears and was highly motivated to have the situation restored.

Patient 2: Person III-30 in the pedigree was a 25-year-old woman (Figure 1). The features and pre-operative hearing thresholds are presented in Tables I and II. Exploratory tympanotomy of the left ear had been performed at the age of 16 years elsewhere owing to congenital conductive hearing loss; it was her wish to have binaural hearing. Disconnection of the incudo-stapedial joint was found, because the long process of the incus was too short. Autologous cortical bone had been used to reconstruct the ossicular chain.

Seven years later, she was referred to the Nijmegen Department of Otorhinolaryngology because of progressive hearing loss. The conductive hearing threshold in her left ear had increased. During exploratory tympanotomy, the autologous incus, which was found to be in its original position, was removed. The incus was normal except for the long process which was much too short. The suprastructure of the stapes was malformed and somewhat curved. The mobility of the stapes in the oval window was considered to be normal. The facial nerve was normal. The autologous incus was transformed and interposed to reconstruct the ossicular chain.
The hearing level did not improve post-operatively (Table IV).

Patient 3: Person II-10 in this pedigree was a 58-year-old woman with unilateral mixed hearing loss (Figure 1). Her syndromal features are shown in Table II and mixed unilateral hearing loss is presented in Table III. She had been experiencing progressive hearing loss in her right ear. As there was a sensorineural component in her hearing loss, a CT scan of the petrosal bones was performed to trace inner ear malformations. No abnormalities were found. Exploratory tympanotomy revealed several congenital anomalies of the ossicular chain and other middle ear malformations; there was a plump incus and the long process of the incus was absent. The stapes suprastructure was also absent and the stapedial footplate was fixed. Besides these ossicular chain abnormalities, aplasia of the round window was suspected. This was in contrast with the CT-scan which demonstrated a normal round window. However also a bony plate could be demonstrated covering the round window (Figure 4).
The facial nerve followed a normal course through the bony canal.
Consequently and in view of a reasonable hearing threshold in her left ear, no attempt was made to improve her hearing (Table IV).

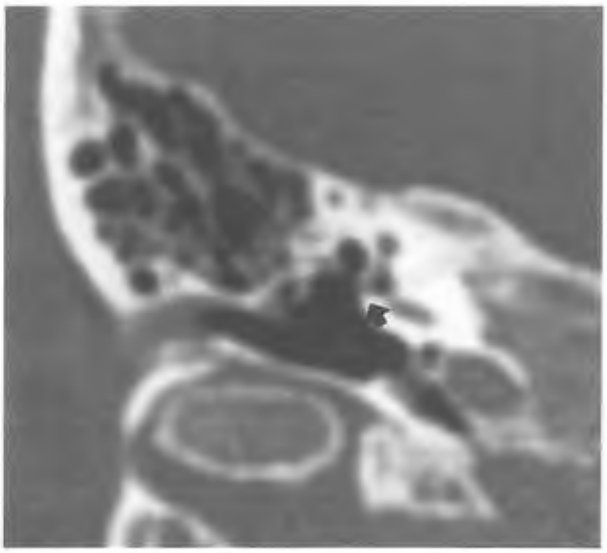

Figure 3: *Axial CT-scan (right ear, patient 3) showing a normal round window covered by a bony plate (arrow).*

Patient 4 (IV-5): This 15-year-old girl was referred to our department at the age of 7 years with complaints of otitis media with effusion. The features are presented in Table II. During follow-up, chronic otitis media developed resulting in bilateral perforation of the tympanic membranes. At the age of 12 years, exploratory tympanotomy and myringoplasty had been performed on her right ear because of serious conductive hearing loss; the ossicular chain and facial nerve were found to be normal. The post-operative results were satisfactory (Table IV). Two years later, the same procedure was performed on the left side. Again, there were no abnormalities besides perforation of the tympanic membrane. Unfortunately, perforation on this side recurred during the first year of follow-up. Her hearing loss was considered to be a residual consequence of chronic otitis media.

Patient 5 (III-6): The bilateral hearing loss of this 36-year-old patient was confirmed by means of audiometry. It was not possible to elicit the stapes reflex on the left side. The medical history indicated chronic otitis media of the right ear and otoscopy revealed perforation of the tympanic membrane. Owing to the presence of perforation of the tympanic membrane in the right ear and the patient's history, it is very likely that the hearing loss was the result of previous otological disease. Therefore, it was unclear whether the hearing loss was caused by a congenital middle ear anomaly, despite the presence of the other features and the absence of a stapes reflex on the left side (Table II). The patient was not troubled by his hearing loss and found it unnecessary to have a hearing aid fitted or to undergo exploratory tympanotomy.

Patients 6, 7 and 8 (III-4, IV-7 and IV-8, respectively) were also found to have hearing loss besides the other syndromal features (Tables II and III). Stapedial reflexes could not be elicited in any of them. Patient 6 had no previous history of otological disease and otological examination dit not reveal any abnormalities besides the preauricular sinus on the left ear. However, his hearing loss was only demonstrated on the right side and he had never considered a hearing aid or surgery. Patients IV-7 and IV-8 (identical twins) were considered to be too young for surgery, so hearing aids were fitted for hearing rehabilitation at the age of 3 years when their hearing loss was first demonstrated. The paediatric audiology unit is currently giving counsel to the children and their parents and has an advisory function with regard to the children's schooling. Both patients had a negative otological anamnesis and history, so their hearing loss can provisionally be attributed to the underlying syndrome in view of the other features present.
High-resolution CT scanning (1 mm slices in coronal and transverse directions) of the inner ears in patients number 1, 3, 5, 6, 7 and 8, showed no abnormalities of the inner ear structures, especially no cochlear dysplasia.
Cases 1 and 5 showed vestibular hyperreactivity of their velocity step (VS) responses, i.e. the initial velocities (V) were too high compared to the confidence limits reported by Theunissen et al.[5] Caloric responses in case 1 were normal. Case 6 had normal VS responses but a marginally pathological caloric side difference of 25% with smaller response on the left side. In all three cases tested, the results of the other vestibular and oculomotor tests were normal.
In the pedigree, 3 other cases with hearing loss are presented (see figure I): cases II-2 (spouse), III-29 and IV-31. The hearing loss of case II-2 was mixed and showed a slow-

ly progressive character. No stapes reflexes could be elicited. The hearing loss was probably based on otosclerosis. The hearing loss in case III-29 and in case IV-31 can be attributed to chronic otitis media. In view of the absence of any other features, these persons appear to be phenotypically unaffected.

Discussion

We consider the syndrome described in this study to be a separate syndrome, despite the similarities with a few documented branchial arch syndromes. The greatest similarities exist between this syndrome and the branchio-oto-renal (BOR) syndrome, referred to in the past as the ear pits-deafness syndrome.[6,7]

The distinction made between the branchio-oto-renal syndrome and the branchio-oto (BO) syndrome in the literature proved to be incorrect because no investigation had been made of the kidneys of the patients who were suffering from the branchio-oto-(renal) syndrome (i.e. the ear pits deafness syndrome).[7,8] In the near future gene-linkage studies are expected to give the final answer to this.

The absence of any renal anomalies in the above-described family and the absence of cervical fistulae or sinuses, and the presence of commissural lip pits, are motives to distinguish this syndrome from the BOR syndrome.

Earlier literature surveys and our own work on the BOR syndrome have shown that only 15% of the persons with the BOR syndrome present with ear pits and deafness without cervical fistulae.[7] The observation that none of the persons in this fully-investigated family comprising 74 members were found to have cervical fistulae, supports the assumption that this anomaly should be differentiated from the BOR syndrome.

The commissural lip pits must be distinguished from the paramedian lip pits which are associated with facial clefting.[9] Commissural lip pits do not generally have any clinical significance. However, in a recent report, mention was made of a unilateral lip pit in combination with an ectopic salivary cyst and an aberrant parotid duct.[10] The morphogenesis of these lip pits cannot be attributed solely to a branchial cleft anomaly. A possible explanation is that only partial fusion took place between the maxillary process and the mandibular process in the 6th to 8th week of embryonal development.

The best known examples of syndromal branchial arch anomalies with associated hearing loss are the Treacher Collins syndrome and the BOR syndrome. Another example is autosomal dominant inherited hemifacial microsomia. The anomalies of the ossicular chain and middle ear associated with these syndromes are generally more severe than those encountered in ears with a congenital middle ear anomaly alone.[11,12] For example, a combination of stapes footplate fixation and the absence of the long process of the incus is often seen and there is a higher incidence of dysplasia of the oval and/or round windows, which is an expression of more extensive branchiogenic involvement. Dysplasia of the inner ear has also been described in the BOR syndrome.[11,13] This can be explained as being the result of impeded growth of the otic capsule, which is surrounded by a layer of cartilage of branchial origin during early development.[14] In the above-described new syndrome, we did not found any evidence of developmental anomalies of the inner ear. The cases we examined did not show any signs of vestibular hypofunction, which was found to be a typical charac-

teristic in half of the cases with the BOR syndrome.[11] However, the number of patients examined (n=3) in this study was too small to draw any definitive conclusions.
The middle ear anomalies revealed by exploratory tympanotomy in our group of patients all showed involvement of the long process of the incus with additional stapes footplate ankylosis in three out of the four operated ears. In one case, dysplasia of the oval window was also present. Stapes surgery produced only one successful long-term result: case III-7, right ear, in whom the piston had been fixed to the incus. In one patient, suspected aplasia of the round window prevented us from attempting to repair the severe congenital ossicular chain anomaly. Malleovestibulopexy achieved only a temporary hearing improvement in one ear; improving the surgical techniques may lead to better results. For example, partly detaching the malleus from the tympanic membrane may be preferable. If the results are not expected to be favourable using this technique, it may even be worthwhile to consider myringochordovestibulopexy, which has recently been introduced.[15] Although the long-term results were favourable in only one out of four ears with ossicular chain anomalies, for the patient in question who was suffering from bilateral hearing loss, this was a major breakthrough. By improving the surgical techniques and gaining experience with such anomalies, success rates can be expected to steadily increase.
The middle ear anomalies described above confirm that the ossicular chain anomalies with associated features in this new syndrome are generally more severe than usual; consequently the chance of successful surgery is lower.[12] In the case of bilateral congenital hearing loss, fitting the patients with hearing aids is still the best solution. Surgery can be considered as an alternative for hearing aids. The chances of achieving a successful result with reconstructive surgery for the treatment of other syndromes with congenital hearing loss and branchiogenic involvement, indicated by features such as pinna dysplasia, eg. the BOR syndrome and the Treacher Collins syndrome, are at present smaller than those for congenital ossicular chain anomalies.[16,17]
In the above-described syndrome it is possible that the middle ear anomalies will be more serious than normal, which may have an unfavourable influence on sucessful surgery. However, this does not mean that the situation is so unfavorable that middle ear surgery should not be considered.

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Chapter Four

The Treacher Collins Syndrome

4.1 The Treacher Collins syndrome. A clinical, radiological and genetic linkage study on two pedigrees*

Abstract

The Treacher Collins syndrome (TCS) is an autosomal dominant hereditary syndrome with variable penetrance and expression. The clinical characteristics are the result of dysmorphogenesis of the first and second embryonal branchial arch systems. The gene responsible has been located on the long arm of chromosome 5. The TCS is rare and in 60% of the patients the family anamnesis is negative. Consequently, only a few family studies are available. This renders it more difficult to make a diagnosis and to comply with the increasing demand for genetic counselling. In order to gain more insight into the diagnosis and variation in expression and penetrance of the TCS, a clinical study was started followed by gene-linkage research. Audiological and physical tests were performed on 59 persons belonging to two families. In selected cases (n=19) vestibular and radiological examinations were also conducted. Blood samples were taken from 55 persons for gene-linkage studies. The diagnosis of the TCS could be made in 13 persons after clinical examination. The radiological detection of zygoma hypoplasia or aplasia played an important supportive role. In addition to the 13 persons with the TCS mentioned above, gene-linkage studies using 8 short tandem repeat polymorphism (STRP) markers, showed positive linkage to chromosome 5q32-33.2 in three persons with clinical non-penetrance. This is the first time that non-penetrance of the TCS has been demonstrated convincingly. In individual cases, clinical examination alone cannot always remove doubts about the diagnosis. Therefore, gene-linkage studies will play a decisive role. Identification of the gene responsible for the TCS is expected to be very useful in clinical practice.

Introduction

The Treacher Collins syndrome (TCS) or mandibulofacial dysostosis is an autosomal dominant inherited syndrome (MIM no. 154500)[1]. The typical features of the TCS, as summarized in Table I, are the result of bilateral morphogenetic disruption of the first

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Table I: *Features of the Treacher Collins syndrome*[2,3,4]

Features of the Treacher Collins syndrome	Frequency
Anti-mongoloid slanting of the eyes	89%
Coloboma, frequently combined with absence of eyelashes on medial part of lower lid	69%
Hypo- or aplasia of the zygomatic arch	89%
Malar hypoplasia	81%
Mandibular hypoplasia	78%
Pinna dysplasia	77%
Conductive deafness, middle ear malformations	50%
Meatal atresia	36%
Cleft palate	28%
Pre-auricular hair prolongation	26%
Absence of lower lacrimal puncta	?
Ear appendages, pre-auricular sinus	?
High arched palate	?
Macrostomia, Malocclusion	?
Nearly missing nasofrontal angle	?
Obstructive sleep apnoe syndrome	?
Choanal atresia	?

and second branchial arches.[2,3,4] Recently, however, unilateral and asymmetrical aspects have been emphasized by Wilkinson[4]. The gene for TCS is localised on the long arm of chromosome 5 [5]. TCS is thought to arise as the result of a de novo mutation in 50% to 60% of cases[2,6]. The incidence is estimated to range from 1 in 40,000 in Japan to 1 in 70,000 live births in Spain[7,8].

Penetrance is thought to be almost 100%. Only occasional cases where non-penetrance is suspected, have been documented[9]. However, in most cases of suspected TCS, careful examination of the obligate carrier frequently reveals minor stigmata of TCS[10].

Mandibulofacial dysostosis bears many eponyms originating from original contributions by Thomson (1847), Berry (1889), Treacher Collins (1900), Franceschetti and Zwahlen (1944) and Franceschetti and Klein (1949)[11-15]. The Anglo-Saxon literature prefers the Treacher Collins syndrome (TCS) as an eponym of mandibulofacial dysostosis.

The TCS can be diagnosed easily on the basis of the clinical appearance in cases with full expression of the syndrome (Figure 1). Consequently genetic counselling can be given without any reticence. However, if only minor stigmata are present, diagnosis and the provision of genetic counselling become more difficult.

The value of genetic linkage studies for individual genetic counselling was evaluated by performing a clinical study on two families with remarkable variation in TCS[16].

Methods

Three persons from the two families were initially known to have TCS. Further examination of family history revealed that there were several other members with a "look alike" appearance in both families. Genealogical study of families A and B resulted in

Figure 1: *Typical appearance of a patient with Treacher Collins syndrome (case IV30 family A).*

two family pedigrees which spanned five generations and three generations, respectively, as illustrated in Figure 2.
A clinical study was launched involving both families, and all the family members were asked to cooperate via the probands. All the subjects underwent detailed otorhinolaryngological assessment. If any of them showed typical features, these were recorded by photography.
All the subjects were tested audiologically by pure-tone audiometry at frequencies from 0.25 to 8 kHz. If conductive hearing loss was present, impedance audiometry (including stapedial reflex) was performed as well.
If TCS was diagnosed or if there was any doubt about the diagnosis of TCS, radiodiagnosis was added to the clinical examination. This consisted of an occipitomental projection of the skull (Water's view, posteroanterior with the cantomeatal line extended 45°, with no inclination of the incident ray) and an orthopantogram.
Special attention was paid to hypoplasia or aplasia of the zygomatic arch, changes in the mandibuls and TMJ abnormalities.
All the cases (except for III13 in family A) who were diagnosed as being affected with TCS based on clinical and radiodiagnostic examination, underwent vestibular tests with electronystagmography. Two cases (II2 and III10, family A) were added to this group because it was very obvious that their offspring were affected. All the cases (n=14) were examined for evoked nystagmus and spontaneous nystagmus in the dark. Smooth pursuit eye movements were tested followed by optokinetic nystagmus

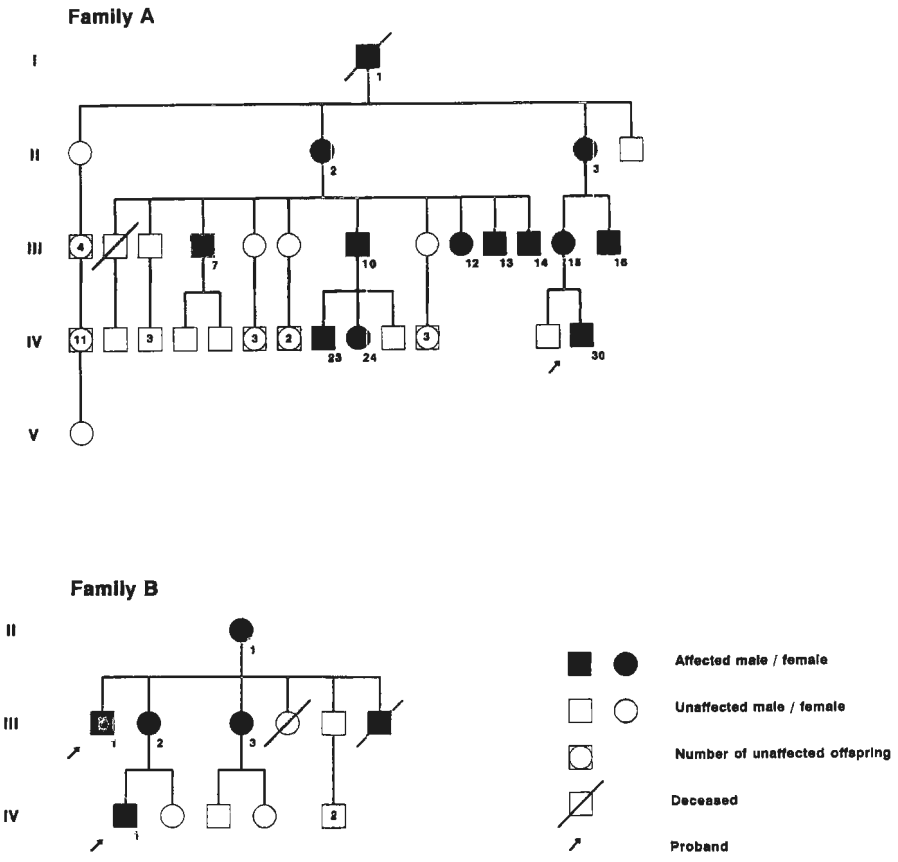

Figure 2: *Pedigrees of family A and family B. All the members cooperated in this study except for case III1 from family B, case IV1 from family A died in early childhood.*

tests. Furthermore the vestibulo-ocular reflex (VOR) was evaluated by using velocity step (VS) tests. Caloric tests were performed only in case IV1 from family B. Methods of calibration, testing and calculation have been outlined previously[17,18].
Blood samples were taken from 55 family members (except for IV18, IV19 IV25 and V1 in family A) and sent to the Manchester Department of Cell and Structural Biology for genetic linkage studies. Methods, analysis and results of this part of the study have been outlined in detail elsewhere[16].

Results

A total of 59 persons from both families participated in this study. One other person known to have TCS (case III1 from family B) was unable to participate.
Clinical examination revealed that 9 persons were affected with TCS (II3, III12, III13, III15, III16, IV24 and IV30 from family A and II1 and IV4 from family B). Five persons had only minor stigmata of TCS (III7, III14, IV2, IV4 and IV5 from family A). It was not

Figure 3: *Case III2 (left) and case III3 (right) of family B. Although both cases are affected no stigmata of the Treacher Collins Syndrome can be demonstrated. However, aplasia of the zygoma arches can be demonstrated by a Water's projection.*

possible to make the diagnosis of the TCS in six persons via clinical examination, although TCS had been demonstrated or was initially suspected in their offspring (II1, II2, III2 and III10 from family A and III2 and III3 from family B, Figure 3). Therefore a total of 14 persons did show (minor) stigmata of the TCS and 6 persons were initially suspected of non-penetrance. The features are summarized in Table II.
In the remaining group of 39 non-affected persons who underwent clinical examination and audiometry, three persons showed abnormalities which were not thought to be related to the TCS. Cases IV9 and IV21 from family A had bilateral commissural lip pits and case III11 had an unexplained sensorineural hearing loss (AD 30 dB, AS 25 dB PTA). This latter (normal) group was excluded from further radiological or vestibular assessment.

Radiology
Conventional X-rays consisting of a Water's projection, were initially taken in 18 persons (Table III). In 2 persons (III2 and III3 from family B) the diagnosis of TCS was strongly supported by the outcome of the Water's projection: aplasia (discontinuity) of the zygomatic arch was visible in contrast with their facial appearance (digital palpation has not been performed). In two other cases (III7 and III14 from family A) who were suspected of having TCS, the diagnosis could be confirmed radiologically. In six cases (II1, II2, III2, III10, IV4 and IV5 from family A) in whom the diagnosis of TCS could

Table II: *Features of the Treacher Collins syndrome present in these families (+ present, ± mild, - not present, right/left).*

Case, M/F, Age	Anti-mongoloid slanting	Coloboma	Malar hypoplasia	Mandibular hypoplasia	Aplasia zygomatic arch	High-arched (HA) or cleft (C) palate	Pre-auricular sinus	Pinna dysplasia	Meatal atresia	Hearing loss
Family A										
II1, F, 81	-/-	-/-	-/-	-	-/-	-	-/-	-/-	-/-	-/-
II2, F, 80	-/-	-/-	-/-	+	-/-	-	-/-	-/-	-/-	+/+
II3, F, 79	+/+	±/±	+/+	+	+/+	-	-/-	-/-	-/-	+/+
III2, F, 56	-/-	-/-	-/-	-	-/-	HA	-/-	-/-	-/-	-/-
III7, M, 49	-/-	-/-	-/-	+	+/+	HA	-/-	-/-	-/-	-/-
II10, M, 45	-/-	-/-	-/-	-	-/-	-	-/-	-/-	-/-	+/-
III12, F, 43	-/-	±/-	+/+	+	+/+	HA	-/-	-/-	-/-	-/-
III13, M, 41	+/+	+/+	+/+	+	+/+	-	-/-	-/-	-/-	-/-
III14, M, 37	-/-	±/±	-/-	+	+/+	HA	+/-	-/-	-/-	-/+
III15, F, 49	-/-	-/-	+/+	+	+/+	HA	-/-	-/-	-/-	+/-
III16, M, 47	+/+	+/+	+/+	+	+/+	HA	-/-	-/-	-/-	+/+
IV2, F, 26	-/-	-/-	±/±	+	-/-	HA	-/-	-/-	-/-	-/-
IV4, M, 24	-/-	-/-	-/-	-	-/-	HA	+/+	-/-	-/-	-/-
IV5, M, 21	-/-	-/-	-/-	-	-/-	HA	+/+	-/-	-/-	-/-
IV24, F, 12	+/+	-/-	+/+	+	+/+	HA	-/-	-/-	-/-	-/-
IV30, M, 20	+/+	+/+	+/+	+	+/+	HA	+/+	+/+	+/+	+/+
Family B										
II1, F, 69	+/+	+/+	+/+	+	+/+	HA	-/-	+/+	+/-	+/+
III2, F, 42	-/-	-/-	-/-	-	+/+	-	-/-	-/-	-/-	-/-
III3, F, 41	-/-	-/-	-/-	-	+/+	-	-/-	-/-	-/-	-/-
IV1, M, 19	+/+	+/+	+/+	+	+/+	C	-/-	-/-	-/-	+/+

Table III: *Results of clinical and radiological examinations (+ yes, - no, ? diagnosis uncertain, N normal, o examination not possible).*

Case, M/F, Age	Clinical diagnosis	Hearing loss	Zygomatic arch	Concavity	Abnormality TMJ	Radiolog diagnosis	Remarks
Family A							
II1, F, 81	-	-	N	-	-	-	
II2, F, 80	-	+	N	-	-	-	
II3, F, 79	+	+	o	o	o	o	discomfort
III2, F, 56	-	-	N	-	-	-	
III7, M, 49	?	-	aplasia	+	+	+	
III10, M, 45	-	+	N	-	-	-	
III12, F, 43	+	-	aplasia	+	+	+	
III13, M, 41	+	-	aplasia	-	-	+	
III14, M, 37	?	+	aplasia	+	-	+	
III15, F, 49	+	+	aplasia	-	-	+	
III16, M, 47	+	+	aplasia	-	-	+	
IV2, F, 26	?	-	o	o	o	o	pregnant
IV4, M, 24	?	-	N	+	-	-	
IV5, M, 21	?	-	N	-	-	-	
IV24, F, 12	+	-	aplasia	+	+	+	asymmetry
IV30, M, 20	+	+	aplasia	o	+	+	
Family B							
II1, F, 69	+	+	aplasia	-	-	+	
III2, F, 42	-	-	aplasia	-	-	+	
III3, F, 41	-	-	aplasia	+	-	+	
IV1, M, 19	+	+	aplasia	-	-	+	asymmetry

not been made on clinical grounds only, no abnormalities could be demonstrated radiologically. The clinical diagnosis of TCS was made in 9 cases as mentioned before and 8 of them underwent radiological assessments: in all of these cases aplasia (discontinuity) of the zygomatic arch was found to be present (Figure 4).
Temporal mandibular joint abnormalities were found in 4 cases, all of whom also had zygomatic arch aplasia (Table III). Concavity of the mandibula was present in 6 cases and corresponded with other radiological signs of TCS in 5 cases. Asymmetrical changes of TCS were found on the conventional X-rays in 2 cases.
In conclusion the combination of clinical examination and radiological assessment only provided a diagnosis in 13 cases. Two other cases without any clinical or radiological signs of TCS (II2 and III10 from family A) could be added to this group because of their affected offspring. In 5 other cases with minimal stigmata, the diagnosis of TCS could not be excluded with certainty.

Audiometry
Pure-tone audiometry revealed an elevated hearing threshold in 9 out of the 20 above-mentioned cases (Table IV). According to the medical history, a congenital etiology was present in all except for two cases (III14 and III15 in family A). Case III14 was suffering from severe life-threatening osteomyelitis and had consequently received

Figure 4: *Aplasia of zygomatic arches on a Water's projection in case III12 family A (arrow).*

ototoxic drugs. Case III15 was suffering from chronic otitis media and otoscopy showed an inflamed perforation of the right tympanic membrane.
The air-bone gap (defined as the mean difference between the air conduction and bone conduction hearing levels at frequencies of 0.5, 1.0 and 2.0 kHz) ranged from 10 to 55 dB. A bone conduction hearing level of more than 25 dB was noticed at some of the frequencies but especially at the higher frequencies (4.0 and 8.0 kHz) in 12 out of the 18 ears (Table IV). After correction for age-related hearing loss, these ears still showed a relevant sensorineural hearing loss[19].
Although stapedial reflex measurements could be performed in 4 cases with a conductive hearing loss of more than 20 dB (4 ears) no reflexes could be elicited.
In the hearing impaired group, 2 patients (3 ears) underwent exploratory tympanotomy. The operative findings and post-operative results of these patients and other patients with the TCS were the subject of a recent study[20].

Table IV: *Hearing thresholds of the TCS patients. First number represents the air-conduction hearing level (dB), the second number the bone-conduction hearing level (dB). When one number is given only the air-conduction was measured.*

		Frequency (kHz)					
Case		0.25	0.5	1.0	2.0	4.0	8.0
Family A							
II2	R	35	35/30	55/40	60/50	70/60	-/-
	L	50	50/35	60/40	75/55	70/50	-/-
II3	R	75	80	90	75	80	-
	L	70	85/50	90/45	85/60	80	-/-
III10	R	75	60/5	50/5	40/15	30/10	75/20
	L	25	20/10	10/5	15/10	30/15	50/20
III14	R	10	10/5	15/10	35/35	50/45	30/30
	L	20	25/5	35/20	30/30	50/35	55/35
III15	R	30	20/0	35/0	25/20	40/25	35/30
	L	20	20	15	20	25	35
III16	R	40	50/10	50/5	50/25	75/25	75/45
	L	55	60/15	65/15	60/35	75/30	70/30
IV30	R	60	60/0	60/0	50/5	45/0	65/15
	L	70	55/5	55/5	55/0	60/5	50/10
Family B							
II1	R	-	95/35	80/25	95/45	95/-	-/-
	L	45	50/15	50/15	40/40	80/50	90/-
IV1	R	60	55/5	50/10	35/15	30/5	45/0
	L	55	50/5	45/5	50/15	35/10	40/15

Vestibular examination

Spontaneous nystagmus of significant intensity (> 6 /s) was not encountered in any of the cases with the TCS. Gaze positions, saccadic and smooth pursuit eye movements and optokinetic nystagmus were normal in all of them. The caloric responses of the proband of family B were normal (16 /s from either side).

Case III14 exhibited strong vestibular hyporeflexia which was close to areflexia. The cervico-ocular reflex (COR), tested in darkness (eyes open) by sinusoidal rotation of the trunk under the head fixed in space at an amplitude of 30 and a period of 10 seconds, showed enhancement typical of that found in labyrinthine-defect subjects[21]. Hyperreflexia of the VS responses was found in 5 cases (II3, III15 and III16 from family A and II1 and III2 from family B).

Gene-linkage studies

Positive gene-linkage could be demonstrated in 16 out of the 55 samples[16]. None of

Figure 5: *Case IV-23. Non-penetrance of Treacher Collins syndrome.*

the 5 cases in whom it was not possible to exclude the diagnosis of TCS with certainty showed positive linkage. However, positive gene-linkage could be demonstrated in the two cases with affected offspring (II2 and III10 from family A) as was expected. Surprisingly one other person who was not suspected of having TCS at all showed positive gene-linkage (Case IV23 in family A, Figure 5). Additional X-rays of this case did not show any abnormalities and the hearing thresholds were normal.
The combination of clinical and radiological assessments and gene-linkage analyses demonstrated that 16 persons were affected with TCS. Only 9 of these cases where initially suspected of having TCS based on the clinical examination alone.

Discussion

The TCS can be diagnosed easily on the basis of the clinical appearance in cases with full expression of the syndrome. Adults with fully expressed mandibulofacial dysostosis have a typical appearance with a convex facial profile and a prominent nose above a retrusive chin. The eyes are deformed by antimongoloid slanting of the palpebral fissures, hypoplastic lower eyelids, partial absence of eyelid cilia and inferolateral dystopia. There are colobomata of the lower lids and lateral canthi, tongues of hair extend onto the cheeks, which are deformed by underlying osseous defects. The external ears may be malformed or malpositioned and hearing can be impaired. The extensive variation in expression is supported by the classification suggested by Franceschetti and Klein, who distinguished between a complete, incomplete, abortive, unilateral and atypical form[15]. In our opinion it would be better to speak in terms of full expression or mild expression. Full expression comprises at least the following features: antimongoloid slanting of the eyes, colobomata and hypoplasia of

the facial bones. If this triad is not present, we consider that the patient shows mild expression. The unilateral or atypical form described by Franceschetti and Klein are more compatible with the oculo-auriculo-vertebral spectrum[22].
In cases where only minor stigmata are present, diagnosis and additional genetic counselling are more difficult. To detect such cases with minor expression of TCS, two Dutch families were invited to undergo selective clinical and radiological investigation combined with gene-linkage studies. During clinical examination we noted remarkable differences in the extent of expression cq. variation in expression within these two families. Radiodiagnostic tests proved particularly useful to confirm the diagnosis of cases who, on clinical grounds, were suspected of having mild expression. We chose two simple radiological examinations which avoided discomfort for the participants and required a minimum of radiation. The most common abnormality and the one most easily recognisable radiologically was zygomatic arch hypoplasia or aplasia. Orthopantograms can be used to demonstrate mandibular hypoplasia (which is difficult to quantify) and changes in mandibular configuration. TMJ abnormalities and asymmetry can be assessed on the same films.
To our knowledge there are no reports in the literature on the radiological screening of persons at risk for TCS. Detailed descriptions have appeared sporadically on TCS-associated deformities found on dried skulls with the help 3-D surface reformation CT scanning and radiocephalometric studies[23-25]. Invariably zygomatic arch hypoplasia was found in these studies. A simple Water's view has proved sufficient to detect this deformity in patients[26]. In the present study the diagnosis of TCS could be confirmed radiologically in all the clinically suspected cases. Furthermore two additional cases with zygomatic arch abnormalities were detected and diagnosed as having TCS mainly on the basis the radiological examination results. Other features, such as TMJ abnormalities and mandibular hypoplasia, were seen less frequently but we were unable to quantify them owing to the method used in this study.
Radiological examination also revealed bony asymmetry in a number of cases, particularly in those with less serious expression of TCS. Similar findings have also been reported in other studies, but usually in cases with full expression of the syndrome[2,24].
Whenever a patient needs any form of surgical treatment CT-scanning is obligatory.
Audiological examination of TC patients is important because about 50% show hearing loss. This is nearly always conductive hearing loss resulting from an ossicular chain anomaly whether or not in combination of aural atresia. It is not recommended to perform reconstructive surgery before the age of ten years[20,27]. Some forms of aural atresia are not suitable for surgery[20].
The sensorineural component in the hearing loss of a few of the above-described cases was unusual, but similar findings have occasionally been reported in other studies[28-30]. There is no explanation for this phenomenon, although some earlier studies described histopathological anomalies of the inner ear[31,32]. One would expect to encounter such findings in cases with the more serious form of TCS and not in the less seriously affected cases.
The finding of vestibular hyperreactivity can be explained in terms of false positivity. Statistical analysis learned that these findings were not significant. In the literature, only an occasional case report presents the results of vestibular investigation of TCS cases, but neither hyporeflexia nor hyperreflexia are mentioned[15,33,34].
So an important observation in this study is that there is no impairment of the VOR in

the TCS. This is contrary to the findings in cases with the Branchio-Oto-Renal (BOR) syndrome who do show hypofunctioning[35]. Sensorineural or mixed hearing loss is also observed more frequently in patients with the BOR syndrome.
With the aid of gene-linkage studies the diagnosis of cases with a clinical c.q. radiological suspicion of TCS could be confirmed in this study. The positive gene-linkage in case IV-23 was a surprise and forms a clinically relevant observation, because non-penetrance of the TCS has never been demonstrated with such clarity before.
In addition, cases II-2 and III-10 from family A showed positive gene-linkage and also non-penetrance. However, the diagnosis of non-penetrance of TCS was suspected on clinical grounds owing to their affected offspring. Because of absence of any trait in these three persons the diagnosis of Treacher Collins syndrome can not be made. It would be better to speack of: affected by the Treacher Collins Syndrome.
A negative family history in 60% of the cases with TCS was always thought to be acceptable. However, the results of this study cause us to doubt whether this is correct. This doubt can also be extended to the incidence of the associated symptoms, as listed in Table I.
The occurrence of non-penetrance and the fact that a number of affected cases had such mild expression, is of great importance for genetic counselling in relation to the Treacher Collins syndrome. By making use of the gene-linkage facilities currently available it will be possible to achieve a greater level of accuracy.

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4.2 Ear Surgery in the Treacher Collins Syndrome*

Abstract

The autosomal dominant hereditary Treacher Collins syndrome manifests itself phenotypically in dysmorphogenesis of particularly the first, but also the second branchial arch system. Consequently, 50% of the patients with Treacher Collins syndrome have a congenital generally pure conductive hearing loss resulting from a major or minor ear anomaly. The outcome of surgery to improve the patients' hearing varies and is sometimes even disappointing. Thorough analysis of 33 cases / 39 operated ears and the strict application of a classification for the anomaly to each ear, enabled us to gain an insight into the most suitable surgical policy and to predict the prognosis of reconstructive ear surgery.

Introduction

The Treacher Collins syndrome (TCS) or mandibulofacial dysostosis is a well-delineated branchial arch syndrome with an autosomal dominant mode of inheritance.[1] However, in about 50% to 60% of the cases the family history is negative and these are thought to be de novo mutations.[2,3]

The Treacher Collins Syndrome is characterized by antimongoloid slanting of the eyes, coloboma of the lower lid, malar and mandibular hypoplasia, hypoplasia or aplasia of the zygomatic arch, cleft palate, external and middle ear malformations and conductive deafness. Less common but associated features have been described too, which are summarized in Table I.

In cases where all the stigmata are present, it is easy to make a diagnosis on the basis of the clinical appearance (Figure 1). The gene responsible for TCS has only recently been localized on the long arm of chromosome 5.[4]

Conductive hearing loss is thought to be present in about 50% of the patients with TCS, which is caused by ossicular chain malformations often in combination with meatal atresia.[2,5] Mixed or sensorineural hearing loss is rare and has only been reported occasionally.[6,7,8,]

Although reconstructive ear surgery can be a challenge for the otologist, the postoperative results are often poor and disappointing.[9]

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Table I: *Features of the Treacher Collins syndrome*

The Treacher Collins syndrome	
Antimogoloid slanting eyes	Absence of eyelashes on medial part of lower lid
Coloboma of the lower lid	Absence of lower lacrimal puncta
Malar hypoplasia	Ear appendages, pre-auricular sinus
Mandibular hypoplasia	Pre-auricular hair prolongation
Hypoplasia or aplasia of the zygomatic arch	Choanal atresia
Pinna dysplasia	Macrostomia
Meatal atresia	Malocclusion
Ossicular chain malformations	Obstructive sleep apnoea syndrome
Cleft palate, high arched palate	

We present a review of the literature on this subject and additionally describe the otological findings and the surgical management of 12 patients with mandibulofacial dysostosis and conductive hearing loss operated on in the Nijmegen Department of Otorhinolaryngology.

Methods

We evaluated the otological data of 12 consecutive Treacher Collins patients operated on at the University Hospital Nijmegen between 1960 and 1990 and reviewed the

Figure 1: *Patient with the typical appearance of Treacher Collins syndrome (case 8).*

literature on ear surgery in TCS patients published since 1960. This review covered only those reports which provided sufficient details to allow description and classification of the anomalies.
Within the total patient group, we made an otological distinction between major ear anomalies (types with at least meatal atresia) and minor ear anomalies (types with only ossicular chain and/or window malformations). Whenever possible, the ear anomaly was further classified according to Altmann (1955) and Cremers and Teunissen (1991), as shown in Table II. If relevant, pinna anomalies were classified according to Meurman (1957, Table II).[10,11,12,13]
The anatomical findings of the ears and the surgical technique applied, for example described in the operation reports or in the published reports, are presented schematically in the tables.
The results of preoperative and postoperative audiological tests are presented as the pure tone average (=PTA; mean thresholds in dB HL at 500, 1000 and 2000 Hz).
In the group with major ear anomalies, 4 of our patients did not undergo reconstructive surgery of the middle ear and aural canal, but they did undergo surgery for the purpose of fitting a Bone Anchored Hearing Aid (BAHA). The anatomical details of the ears of these patients were obtained by means of radiodiagnostics using high resolution CT scanning. Radiodiagnostic data were also available for the remaining patients, but as the methodology varied considerably over the 30 years period, these have been omitted here.

Table II: *Classification of the pinna anomaly, major ear anomaly and minor ear anomaly. According to Altmann (1955), Cremers et al (1984), Cremers and Teunissen (1991) and Meurman (1957).*[10-13]

	Major anomaly[10,11]
I	Meatus is small and frequently only present in its medial portion.
IIA	Total bony atresia over only part of the length of the meatus, or the canal is partially aplastic.
IIB	Total bony atresia over the full length of the meatus. The tympanic cavity may be smaller than normal.
III	An absent external meatus and an either small or missing tympanic cavity.
	Minor anomaly[12]
I	Isolated stapes ankylosis.
II	Stapes ankylosis with associated anomaly of the incus and/or malleus.
III	Mobile stapes footplate, but anomalous or fixed incus and/or malleus.
IV	Aplasia or dysplasia of the oval and/or round window.
	Pinna anomaly[13]
I	The pinna is smaller, rudimentary and often located in an abnormal position. The different parts of the pinna are still discernable.
II	The pinna, besides being smaller and often in an abnormal position, is represented by a vertical curving ridge, resembling a primitive helix.
III	The rudiment of the pinna has no resemblance to any portion of the normal pinna.

Table III: *Patient related items. Age at time of surgery.*

Case	Age (yrs)	Sex	Author	Case	Age (yrs)	Sex	Author
1	16	m	Marres (1994)	18	11	f	Fernandez (1964)
2	12	m	,,	19	3	f	,,
3	10	f	,,	20	35	m	Cummings (1965)
4	21	m	,,	21	16	m	Plester (1961)
5	16	m	,,	22	12	f	Ombrédanne (1970)
6	16	f	,,	23	35	m	Edwards (1964)
7	18	m	,,	24	33	f	Fernandez (1964)
8	37	m	,,	25	6	f	,,
9	5-23	f	,,	26	23	f	Gerhardt (1970)
10	33-36	m	,,	27	36	m	Holborow (1961)
11	7	f	,,	28	31	f	,,
12	38	f	,,	29	8	m	Fernandez (1964)
13	1	f	Gill (1969)	30	11	m	,,
14	13	m	,,	31	31	m	Herberts (1962)
15	7	f	Herberts (1962)	32	15	m	Ombrédanne (1970)
16	19	m	Ombrédanne (1966)	33	8	m	,,
17	8	f	Fernandez (1964)				

Results

From the 12 patients in our own material, 8 cases had major ear anomalies (Table IV_A, cases 1-4 and Table VIII, cases 5-8, page 100 and 106). Case 3 had asymmetrical ear anomalies: her left ear was classified in the major anomaly group and her right ear in the minor anomaly group.
Four patients had bilateral minor ear anomalies (Table VI_A, cases 9-12).
In the literature, only 11 out of the 27 reports on ear surgery in relation to the Treacher Collins syndrome described the individual patients in sufficient detail to be included in this study.[14-24] Tables IV_B, V, VI_B and VII (pages 101-105) list the otological findings, surgical techniques and postoperative results of the patients described in the literature (cases 13-33, 21 patients / 28 ears). Patient related items as age at time of surgery and sex are summarized in Table III.

Major ear anomalies
The middle ear could not be identified during surgical exploration in 6 out of the 14 ears with a major anomaly summarized in Tables IV_A en $_B$. Although a middle ear cavity was present in the remaining eight ears, it was found that both the malleus and incus were either absent or seriously malformed, or fused and fixed in the epitympanum. The stapes was abnormal in 4 out of the 7 reported ears and in two ears it was absent. In cases 1, 4 and 19 a cholesteatoma was present.

Minor ear anomalies
The data on 25 ears with a minor ear anomaly (Tables V-VII) showed that 14 ears had a Type III minor ear anomaly: mobile (malformed) stapes but an anomalous or ankylotic incus and/or malleus (Figure 2). The malleus had a normal shape in only 4 out of

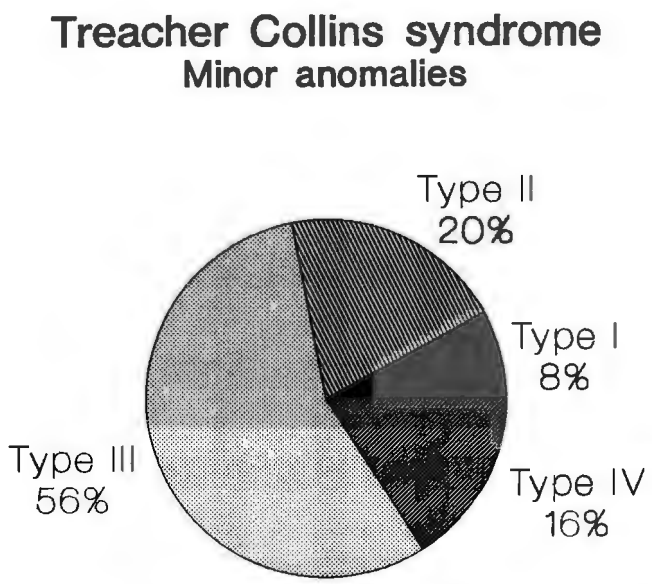

Figure 2: *Overview of the type of middle ear anomaly as defined in Table II of 25 ears with only congenital middle ear anomaly in patients with Treacher Collins syndrome (total series).*

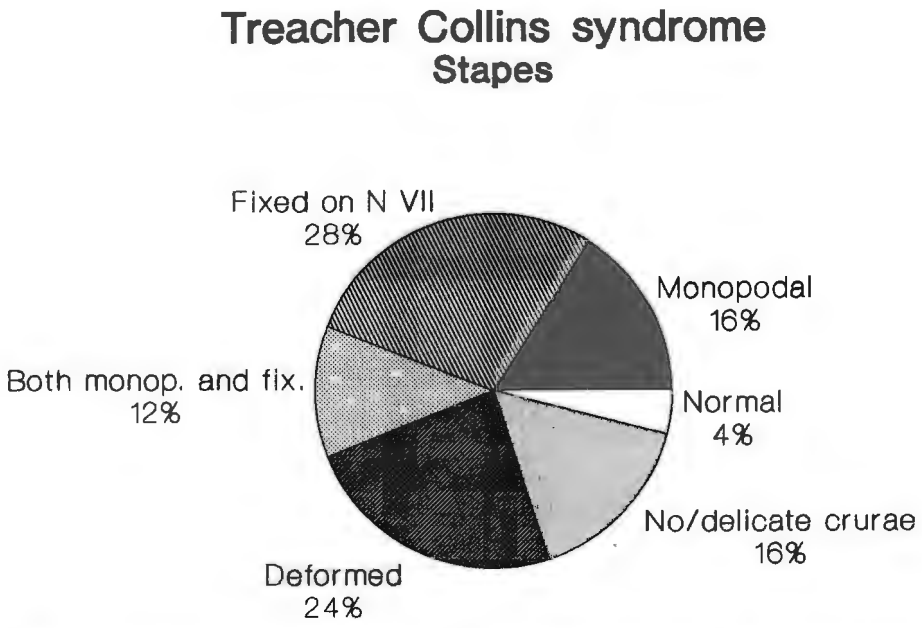

Figure 3: *Description of the stapes anomalies in 25 ears with only congenital middle ear anomaly (total series).*

the 25 ears. In 3 cases, data on the malleus were lacking. The malleus was absent in 7 ears. Fusion between a malformed incus and malleus was found frequently, whether or not in combination with fixation in the epitympanum.
Only one patient had a normal incus (2 ears). In the majority of cases the incus was seriously malformed or even absent. Sometimes the incus appeared to have been replaced by a bony strand.
The stapes was abnormal in 24 out of the 25 ears with a minor ear anomaly (Figure 3). The stapes was monopodal in 7 ears (Figure 4). In 40% of the ears with a minor anomaly, the stapes was fixed to the facial canal, or was curved against the facial nerve. An ankylotic footplate was found in 28% of the minor ear anomalies.

Oval window, round window, facial nerve
In the two groups of patients with a major or minor ear anomaly, the oval window was generally smaller than normal and there were two cases of aplasia. The round window was aplastic in 6 ears.
No records were available concerning the facial nerve in 8 out of the 25 ears with a minor anomaly. Only three out of the 17 remaining ears had a normal facial nerve and in many cases it was bare throughout the cavum tympani. In at least 7 ears, the oval

Figure 4: *Monopodal stapes in TCS.*

window was partly covered by the facial nerve. Three of the cases with a minor anomaly had an unusually thick chorda tympani.

Post-operative hearing and surgical complications
Hearing improvement was achieved in only 5 out of the 14 above-described ears with a major anomaly. The average improvement was 30 dB (range 20-40 dB); a postoperative PTA ≥ 30 dB was found in three cases.
In 20 out of the 25 ears with a minor anomaly, there was a hearing improvement of ≥ 10 dB. The average hearing improvement in these 20 ears was 25 dB (range 10-60 dB); a postoperative PTA of 30 dB or less was found in only 10 ears. No hearing improvement was achieved in 5 out of the 25 (20%) ears with a Type I, II, III or IV minor anomaly.
When the *patients* were divided into groups on the basis of their age at surgery, it was found that the results of the patients who were older than 10 years of age (n=13, mean hearing gain 23 dB, SD 18 dB) were better than those of the patients 10 years of age or younger (n=6, mean hearing gain 9 dB, SD 8 dB), however, the difference was not significant (Student's t= 1.78, 17 degrees of freedom, p> 0.05).
The type of minor ear anomaly was not clearly related to the surgical result: types I and II showed a mean hearing gain of 17 dB (n=6), whereas types III and IV were associated with a mean improvement of 18 dB (n=14).
It was striking that none of the studies reviewed above mentioned a postoperative increase in the perceptive threshold or postoperative facial palsy and similarly, none of the patients in our series suffered from these complications. Partly to avoid these complications, the operation was unsuccessful and had to be terminated in 20% of a total of 39 operated ears with an anomaly.

Bone Anchored Hearing Aid
Owing to the severity of the anomaly, we recently decided not to perform reconstructive surgery on four of our own patients with a major ear anomaly Type III (cases 5, 6, 7 and 8). However, they were considered to be suitable candidates for a bone anchored hearing aid (Table VIII) and underwent surgical implantation of a percutaneous titanium screw which was anchored in the temporal bone (Figure 5); at a later date the patients were fitted with the BAHA which was attached to the implanted screw. The surgical procedures used have been described in detail by Tjellström (1985).[25] The total follow-up period of these four patients was 80 months. Adverse skin reactions around the implant occurred three times but could be treated successfully with antibiotic ointment. Two months after implantation, the patients underwent audiological examinations to compare their performance with their previous conventional bone conduction hearing aid (CBHA) to their performance with the BAHA. The sound field warble tone threshold test showed lower thresholds with the BAHA especially at the higher frequencies. The average threshold shift was -7 dB (range +2 to -15 dB) per frequency (0.25 to 8 kHz). The sound field discrimination task in quiet could not differentiate between the two hearing aids, as the maximum phoneme score was 100% with both hearing aids in all the patients. The speech/noise ratio with the Plomp test improved with the BAHA by an average of 2.1 dB (range 0.5 to 4.4 dB) which is the equivalent of a 35% improvement in the speech score in noise.[26] In two of the four patients, this improvement was statistically significant.

Figure 5: *Bone Anchored Hearing Aid, detail (case 8).*

Discussion

Major ear anomalies

Atresia of the aural canal in patients with the Treacher Collins syndrome in combination with dysplasia of the pinna, forms a serious functional and cosmetic problem. Meatus atresia is found in 36% of the affected persons.[27] This percentage is probably on the high side because the variation in expression of Treacher Collins syndrome has so far been underestimated.[28] The majority of cases have a Type III anomaly and radiological examinations nearly always show the absence of a middle ear, which represents the most complicated major ear anomaly. Besides meatus atresia, the Treacher Collins syndrome also has other characteristic abnormalities, such as the absence of mastoid pneumatisation, an anteriorly placed mastoid segment and consequently an anteriorly placed sigmoid sinus, a low-lying tegmen and in accordance with the classification of Type III, a slit-like or absent middle ear cavity.[29,30] However, the meso-

tympanum and hypotympanum are more normal than the epitympanum (especially if the external meatus is present).[31] Recent studies have shown a bony cleft in the lateral aspect of the temporal bone just anterior to the mastoid.[9] This finding may be clinically relevant because it is likely that it contains the facial nerve, which generally runs an abnormal and more anterior course from the geniculate ganglion. Sometimes dehiscence of the canal in the middle ear is observed.
Although not typical for the Treacher Collins syndrome, a congenital cholesteatoma was found in 3 ears out of a total of 14 operated ears with a major anomaly. Two of these ears had a bony atresia. This finding is not unusual in cases with congenital aural atresia. In a series of 62 ears with partial or total atresia, 8% were found to have a cholesteatoma.[32] However, in our own patients, this never formed the primary indication for surgery. There is no evidence in the literature that the presence of a congenital cholesteatoma without symptomology should form a clear indication for surgery within this patients group.
Computed Tomography makes it possible to classify aural atresia and now forms an essential part of the pre-operative diagnostic procedure.
The abnormalities in Treacher Collins patients with a major ear anomaly are so complex that in our opinion there is seldom a good indication for reconstructive surgery. This is particularly the case with Type II and Type III (aural atresia) anomalies. However, our own experience and that of other authors has shown that it is possible to achieve positive results in selected cases. In our opinion, there is a better alternative for the majority of patients nowadays as they can be fitted with a CBHA or a BAHA. With the CBHA problems are frequently encountered in relation to the fitting of the aid, the cosmetic appearance, skin irritation as a result of the constant pressure applied to the mastoid process and the lack of support for the hearing aid owing to microtia, as was the case in patients 6 and 7 (Table VIII). Four of our patients were fitted with a BAHA because they experienced these problems with the CBHA. All the patients can wear their new hearing aid all day and prefer it to the CBHA. The most important advantages reported by the patients are better speech intelligibility in noise and an improved aesthetic appearance.

Minor ear anomalies
If a patient presents with a congenital anomaly of the middle ear only, reconstructive surgery can be considered if he or she is older than 10 years of age.[12] The preoperative diagnostics comprise otoscopy, pure-tone and speech audiometry, tympanometry and HR CT scanning of the temporal bones to evaluate the middle ear, its contents and the surrounding structures. Besides the necessary experience of the otologist, the anaesthesist should also have experience with the specific perioperative complications within this patients group, such as potential problems with airway management.[33]
The surgical findings which can be expected during exploratory tympanotomy are diverse, such as the absence of (part of) the ossicular chain, severe deformity of the ossicular chain, ankylosis of the malleus and/or incus and/or stapes footplate, monopodal stapes, oval window partially covered by the facial nerve and close contact between the stapes and the facial nerve.
The disappointing results of reconstructive surgery in TCS patients are generally caused by a deformed stapes. If the stapes is mobile but the stapes head is bent to-

wards the facial nerve, this will impede successful ossicular chain reconstruction. We no longer perform stapes mobilisation in the case of ankylosis of the footplate or fixation to the facial canal in view of the poor long-term follow-up results. In these situations, new techniques should be applied. One such technique involves making a stapedotomy opening in the footplate and subsequently removing the stapes suprastructure using an Argon laser. Functional ossicular chain reconstruction can be achieved by malleo-vestibulo-pexy, as was described by Edwards and Cummings.[17,19] Fixation of a piston to the chorda can be carried out if the malleus handle is absent and then myringo-chordo-vestibulo-pexy can be created by interpositioning cartilage between the chorda and the tympanic membrane.[34] Whether these new techniques lead to the desired level of hearing gain should become clear in the near future.

Conclusions

The early detection of (possible) hearing loss in TCS patients is of great importance in order to be able to rehabilitate their hearing with a hearing aid. Classifying an ear with aural atresia has proved to be worthwhile so that it can be estimated whether reconstructive ear surgery will be sufficiently successful. Computed tomography of the ear is a necessity in this respect. The diagnosis of TCS in combination with congenital aural atresia appears to predict fairly frequently that the degree of malformation of the middle ear will be so severe that reconstructive surgery of the middle ear function will only be successful in exceptional cases. Rehabilitation with a CBHA or a BAHA generally takes preference above reconstructive ear surgery.
Similarly, if the patient has a minor ear anomaly, the hearing loss will be so severe that rehabilitation should be started at the earliest possible opportunity with an air conduction hearing aid. However, the patient's hearing can be improved surgically, but it is best to delay the operation until he or she has reached the age of 10 years. The chances of success are lower than usual because of the presence of associated anomalies of the ossicular chain. In addition, patients with TCS have specific abnormalities of the stapes which impede successful reconstruction of the ossicular chain. The facial nerve often runs a deviant course which involves the risk of damage during surgery. The application of the new surgical techniques requires extra expertise from the surgeon.

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Table IV A: *Operative findings and results in patients with major ear anomalies, Nijmegen series (Table IV B: series from the literature). Hearing thresholds in all tables throughout: first number represents air conduction hearing level, second number represents bone conduction hearing level in dB (both expressed as the mean at 500, 1000 and 2000 Hz). When only one number is given, this represents the air conduction level (N= normal, NR= not reported).*

Case	Ear	Type	Ossicular chain	Oval and round window	Facial nerve	Type of surgery	Remarks	Preoperative hearing	Postoperative hearing
1	left	I	malleus and incus malformed fixed in epitympanum, stapes mobile, bony stapedial tendon to the incus	N	N	canalplasty, myringoplasty, ossicular chain reconstruction with autologous ossicles	congenital cholesteatoma	60 / 0	20 / 0
2	right	III	middle ear cavity not identified					65 / 15	65 / 15
3	left	III	middle ear cavity not identified					50 / 5	50 / 5
4	right	IIA	malleus, incus, stapes fused into one rudimentary piece	NR	bare, normal course	mastoidectomy, tympanoplasty Type IV	congenital cholesteatoma	60 / 0	60 / 0

Table IV B: *Operative findings and results in patients with major ear anomalies, series from the literature.*

Case	Ear	Type	Ossicular chain	Oval and round window	Facial nerve	Type of surgery	Remarks hearing	Preoperative hearing	Postoperative
13	right	III	middle ear cavity not identified					NR	
	left	III	no malleus+incus found, stapes grossly deformed	NR	deformed	exploration		NR	
14	right	III	middle ear cavity not identified					NR	
	left	III	middle ear cavity not identified					NR	
15	un-known	II/III	malformed caput, manubrium absent, malformed incus, mobile stapes	N	bare	minor open cavity, tympanoplasty		65	40
16	right	III	absent, except rudimantary caput malleus	aplasia	NR	fenestration	small middle ear cavity	70 / 15	50 / 15
	left	III	atrophic malformed incus, rudimentary caput malleus, stapes absent	aplasia	NR	fenestration		60 / 10	30 / 10
17	left	III ?	incus+malleus fused into one piece, stapes NR	NR	NR	mastoidectomy, tympanoplasty Type III	no follow-up	55 / 5	
18	left	III	middle ear cavity not identified					NR	
19	left	I	malleus+incus absent, stapes deformed, no crurae	OW covered by bare	N VII	mastoidectomy, fenestration, tympanoplasty Type IV	small attic cholesteatoma	65 / 5	30

Table V: *Operative findings and results of patients with minor ear anomalies Types I and II, series from the literature.*

Case	Ear	Type	Ossicular chain	Facial nerve	Type of surgery	Remarks	Preoperative hearing	Postperative hearing
20	left	II	fused malformed incus and malleus, caput of stapes fixed on processus cochleariformis, crurae very delicate, ankylotic footplate	N	stapedectomy, wire prothesis from handle of malleus to vestibule	chorda encased in bony plate	90 / 35	90 / 35
			re-exploration: extrusion of wire through eardrum, wire repositioned				90 / 35	45 / 35
21	right	I	malleus, incus normal, monopodal ankylotic stapes, fixed onto facial canal	NR	stapes mobilisation		55/ 0	15 / 0
	left	I	malleus, incus normal, monopodal ankylotic stapes, fixed onto facial canal	NR	stapes mobilisation		40 / 0	20 / 0
22	left	II	short handle of malleus, fixed incus with delicate long process, monopodal ankylotic stapes	bare	stapedectomy, vein graft on oval window, autologous incus interpositioning to handle of malleus		60 / 0	30 / 10
23	right	II	malleus NR, long process of incus delicate and fibrous distally, monopodal stapes with restricted mobility	NR	stapedectomy, vein graft on oval window, polyethylene strut (6 mm) to neck of malleus		65 / 10	25 / 10
24	right	II	malleus, incus absent, stapes deformed, ankylotic footplate	N	tympanoplasty Type III		70 / 20	70 / 20
25	right	II	malleus, incus absent, stapes no crurae, ankylotic footplate	bare	expl. tympanotomy only	very large chorda tympani	60 / 0	60 / 0

Table VI A: *Operative findings and results of patients with minor ear anomalies Type III, Nijmegen series (Table VI B: series from the literature).*

Case	Ear	Type	Ossicular chain	Facial nerve	Type of surgery	Remarks	Preoperative hearing	postoperative hearing
3	right	III	malleus + incus fused, fixed in epitympanum, no caput mallei, long process malformed, rudimentary mobile stapes	NR	ossicular chain reconstruction		50 / 10	35 / 5
9	right	III	malformed malleus, incus absent, bony strand instead, rudimentary mobile stapes in contact with facial canal	bare	ossicular chain reconstruction with autologous cartilage	marked thickening of chorda	50 / 5	40 / 5
			re-exploration: interpositioning of transformed incus between stapes and chorda tympani				40 / 5	40 / 5
	left	III	homologous fixed malformed malleus, rudimentary incus, stapes fixed onto facial canal, anterior crus absent	bare	mobilisation of stapes, iIncus transpositioning between footplate and eardrum, malleus handle separated from fixed caput		55 / 0	40 / 0
			re-exploration: interpositioning of homologous incus between stapes and eardrum				50 / 5	30 / 5
10	right	III	malleus normal, rudimentary incus with fragile long process fixed in epitympanum and onto facial canal, malformed mobile stapes	N	autologous incus repositioning between stapes and eardrum		50 / 10	40 / 15
	left	III	malleus normal but fixed, incus malformed and fixed, long process rudimentary and in contact with facial canal, stapes	abnormal branch	homologous corpus mallei interpositioning between stapes and eardrum, tympanoplasty	thickening of chorda	40 / 20	25 / 5
11	left	III	malleus + incus absent, bony strand between annulus and rudimentary stapes suprastructure, crurae absent, mobile footplate	bare, covering oval window	exploratory tympanotomy, terminated		55 / 10	50 / 5
12	right	III	malleus normal, incus absent, stapes curved against facial canal	covering oval window	homologous corpus mallei interpositioning between stapes and eardrum	chronic otitis media	45 / 10	45 / 10

Table VI B *Operative findings and results of patients with minor ear anomalies Type III, series from the literature.*

Case	Ear	Type	Ossicular chain	Facial nerve	Type of surgery	Preoperative hearing	Postoperative hearing
26	right	III	fused rudimentary malleus and incus, stapes fragile and monopodal	bare, partially covering oval window	autologous incus-malleus interpositioning between footplate and eardrum	55 / 0	25 / 0
27	left	III	malleus NR, incus absent, thin strand between flattened stapes and malleus	NR	polythene prosthesis interpositioning between stapes and eardrum	75 / 10	15 / 10
28	left	III	malleus NR, incus absent, stapes monopodal	NR	polythene prosthesis interpositioning between stapes and eardrum	55 / 10	25 / 10
29	right	III	malleus + incus malformed, malformed stapes fixed onto facial canal	partially covering oval window	polyethylene prosthesis interpositioning between stapes and tympanoplasty Type III	65 / 0	gain: nil
	left	III	malleus + incus absent, stapes deformed	partially covering oval window	polyethylene prosthesis interpositioning between stapes and tympanoplasty Type III	45 / 5	gain: nil
30	right	III	rudimentary malleus, incus malformed, malformed stapes against facial canal	partially covering oval window	tympanoplasty Type III	65 / 10	45
31	NR	III	absent caput mallei, malformed incus, absent footplate and crurae	NR	fenestration of posterior canal	60	45

Table VII: *Operative findings and results of patients with minor anomalies Type IV, series from the literature.*

Case	Ear	Type	Ossicular chain	Facial nerve, oval and round	Type of surgery window	Preoperative hearing	Postoperative hearing
22	right	IV	fused malleus and incus, rudimentary caput mallei, incus fixed in aditus, long process absent, malformed stapes crurae, ankylotic footplate	bare facial nerve, partially covering normal oval window, aplasia of round window	stapedectomy with vein graft on oval window and reinterpositioning of stapes, creation of neo-round window	55 / 10	20 / 10
32	left	IV	malleus absent, corpus incudis rudimentary, stapes bent over facial nerve, small footplate	facial nerve: NR, small oval window, aplasia of round window	stapectomy, creation of neo-round window, interpositioning of bone chip	65 / 25	50 / 25
33	right	IV	malleus absent, incus malformed, long process absent, minuscule stapes remnant partially covered by facial nerve	bare facial nerve covering small oval window, round window absentwith skin graft	stapectomy, creation of neo-round window, tympanoplasty Type IV	75 / 15	55 / 15
	left	IV	malleus + incus, minuscule stapes remnant partially covered by facial nerve	bare facial nerve, small oval window round window absent	stapectomy, creation of neo-round window, tympanoplasty Type IV	60 / 25	40 / 25

Table VIII: *CT scan findings of four patients with major anomalies who were fitted with a BAHA (from the present series).*

Case	Ear	Pinna	Meatus	Mastoid	Middle ear cavity and ossicles	Facial nerve	Inner ear, round and oval window	Hearing threshold (dB)
5	right	I	II A	no pneumatisation, hypoplastic	aerated slit-like cavity, rudimentary ossicles in the epitympanum	aberant course of descending part	N	65 / 5
	left	I	II A	symmetry				55 / 0
6	right	III	III	no pneumatisation, severe hypoplasia	very narrow slit-like cavity, filled with soft tissue, fixed rudimentary ossicles	aberant course of descending part	small lateral semi circular canal, wider than normal	75 / 15
	left	III	III	NR				75 / 5
7	right	II	III	no pneumatisation, hypoplastic	aerated slit-like cavity, ossicles absent	aberant course of descending part	N	60 / 0
	left	III	III	symmetry				55 / 5
8	right	II	II A	no pneumatisation, hypoplastic	aerated slit-like cavity, ossicles absent	not visualized	not visualized	75 / 15
	left	II	I	symmetry				70 / 10

4.3 Treacher Collins syndrome: Correlation between clinical and genetic linkage studies*

Abstract

Treacher Collins syndrome (TCOF1) is an autosomal dominant disorder of craniofacial development in which there is considerable variability in the clinical manifestations. The TOCF1 locus has previously been mapped to chromosome 5q32-33.2 and markers flanking the disease locus identified.
In the current investigation we have analysed 8 short tandem repeat polymorphisms for linkage to TCOF1 in a large family with multiple affected individuals. Linkage analysis suggested that TCOF1 in this family was linked to markers in the region 5q32-33.2. We have used the results to make diagnostic predictions in certain mildly affected and apparently unaffected individuals.

Introduction

Treacher Collins syndrome (TCOF1) is an autosomal dominant disorder of craniofacial development, which has an incidence of approximately 1/50000 live births. The clinical features include (1) abnormalities of the pinnae (external ears), which are frequently associated with atresia of the external auditory canals and anomalies of the middle ear ossicles (bilateral conductive hearing loss is therefore common),[1] (2) hypoplasia of the facial bones, particularly the mandible and the zygomatic complex, (3) downward slanting of the palpebral fissures with colobomata (notching) of the lower eyelids and a paucity of lid lashes medial to the defect, and (4) cleft palate.[2,3]
Whilst expression of the TCOF1 locus is extremely variable the clinical features are usually bilaterally symmetrical.[4] Some individuals are, however, so mildly affected that it is difficult to reach a clinical diagnosis and provide accurate genetic counselling. The mutated gene occasionally appears nonpenetrant, although, in most cases where this is suspected, careful clinical and radiological examination of the obligate carrier frequently reveals minor stigmata of TCOF1.[5] In addition, only 40% of cases have a previous family history, the remaining 60% appear to arise as the result of *de novo* mutations.[6]

***MJ Dixon, HAM Marres, SJ Edwards, J Dixon, CWRJ Cremers. Clinical Dysmorphology 1993: in press.**

The gene mutated so as to cause TCOF1 was initially mapped to the long arm of chromosome 5 at 5q31-34.[7] This localisation was subsequently confirmed by Jabs et al.[8] More recently, a number of highly informative short tandem repeat polymorphisms (STRPs) have been identified in the vicinity of the TCOF1 locus, which have permitted the refinement of its localisation to 5q32-33.2 and the identification of close flanking markers for the disorder.[9,10] All the families that have been analysed to date (approximately 30 families) suggest linkage of the disease locus to markers in the same region of the genome with none showing unequivocal evidence of nonlinkage, these data supporting genetic homogeneity. In the present investigation we have utilised the high density of highly informative STRPs in the region of the TCOF1 locus to make diagnostic predictions in certain mildly affected and apparently unaffected individuals.

Subjects and methods

Pedigrees

The pedigree of the family assessed in the current study is presented in figure 1. All patients were examined clinically, radiographically and audiometrically and scored as affected if they presented with the clinical signs noted above. Venous blood samples were taken with informed consent from 26 individuals of whom 12 were affected. Where possible, paternity was confirmed genetically.

DNA analysis

The STRPs used in the current study are presented in Table I. The utilisation of these STRPs for genotyping has been described previously.[9,10] Negative controls were established for all reactions.

Linkage analysis

The alleles were scored and the data coded in linkage format. To facilitate the linkage analysis, the genotype results were processed to produce a maximum of four alleles at each marker locus, while preserving linkage information, as described by Ott.[11] The TOCF1 locus was modeled as an autosomal dominant, two allele system. The gene frequency and the penetrance in heterozygotes were taken as .00001 and .99 respectively. Simulations have previously shown that altering the penetrance between .90 and .99 makes little difference to the results obtained.[5] Pairwise analysis was performed using the MLINK routine of the LINKAGE package.[12,13] Maximum-likelihood estimates of sex-averaged recombination were calculated using ILINK. Significance was evaluated using the standard criterion ($Z > 3.0$).

Results

The detailed results of the clinical, radiological and audiological investigations are presented in Marres et al.[14] On clinical examination 7 patients exhibited obvious clinical signs of TCOF1 (II.5, III.9, III.10, III.13, III.14, IV.4 and IV.6), while an additional 2 patients (III.2 and III.11) exhibited only minor stigmata (Figure 2). In two additional patients (II.3 and III.6) a diagnosis of TCOF1 could not be made on clinical grounds alone

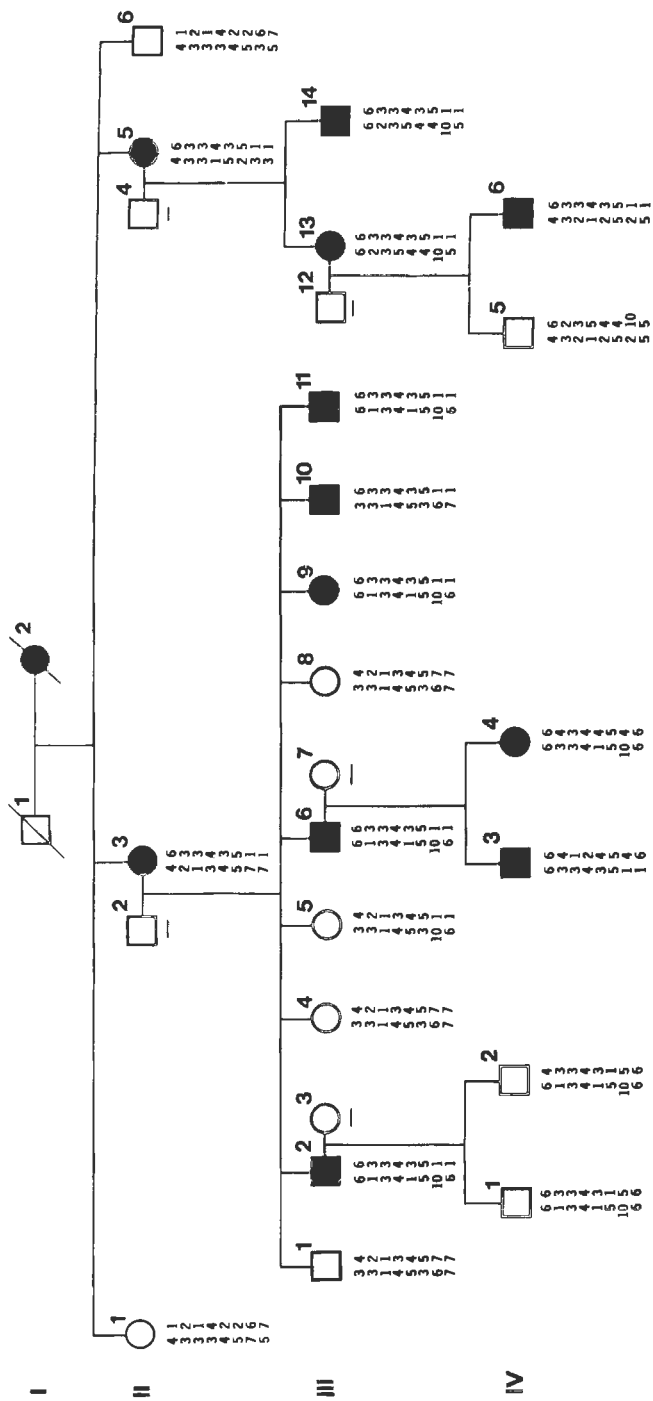

Figure 1: *Partial pedigree of the family. The genotypings for the 8 STRPs used in the present study are indicated. They are (from top) 2C7 (D5S365), E5.12 (D5S373), 2D10 (D5S376), 90-1 (D5S519), SPARC (SPARC), MFD116 (D5S209), IG83 (D5S528) and IG52 (D5S527).*

Table I: *Description of linkage markers.*

Probe	Locus	Heterozygosity	Reference
2C7	D5S365	0.76	Dixon *et al*, 1992
E5.12	D5S375	0.70	Dixon and Dixon, 1993
2D10	D5S376	0.74	Dixon *et al*, 1992
IG90	D5S519	0.72	Dixon *et al* 1993
SPARC	SPARC	0.80	Dixon *et al*, 1993
MFD116	D5S209	0.71	Weber *et al*, 1991
IG83	D5S528	0.85	Dixon and Dixon, unpublished
IG52	D5S527	0.89	Dixon *et al*, 1992

(Figure 3). In both cases the patients had produced offspring who were diagnosed as being clinically affected. Radiographic examination was not able to confirm whether or not these patients were affected, however, in individuals III.2 and III.11 the clinical diagnosis was confirmed, both patients exhibiting hypoplasia of the zygomatic complex. In total, it was therefore possible to diagnose 11 patients as being affected either clinically and/or radiologically.

The results of the genetic linkage studies are presented in Figure 1 and Table II. In total 8 STRPs were analysed for linkage to TCOF1 in this family. This data indicates that TCOF1 in this family is linked to all of the markers analysed suggesting that mutations in the same gene are responsible for causing TCOF1 in this family as in those previously analysed.[9,10] A combination of physical and genetic mapping in 22 TCOF1 pedigrees has previously suggested that the order of these markers is:
2C7--E5.12--2D10--IG90--TCOF1--SPARC--MFD116--IG83--IG52.[9,10] The results of marker-marker pairwise linkage analysis derived from these 22 TCOF1 pedigrees suggests that the most proximal of these markers (2C7) and the most distal (IG52) are linked (Zmax = 4.99, 0 = θ.139). This genetic distance equates to approximately 14 Mb of DNA.

The results of the linkage analysis support the affected diagnosis made in those individuals who exhibited only very minor stigmata of TCOF1 (III.2 and III.11), neither of whom have clinically affected offspring, and in individual III.6 (who has a clinically affected daughter, IV.4), as all three individuals inherited the affected allele in 6 out of

Table II: *Pairwise Lod scores for TCOF1.*

		Lod score at a recombination fraction of								
Probe	Locus	0.00	0.5	0.10	0.15	0.20	0.30	0.30	Zmax	θ
2C7	D5S365	3.89	3.55	3.20	2.83	2.44	1.59	0.66	3.89	0.00
E5.12	D5S373	3.79	3.45	3.10	2.75	2.38	1.59	0.72	3.78	0.00
2D10	D5S376	2.15	1.95	1.75	1.54	1.33	0.85	0.34	2.15	0.00
90-1	D5S519	3.39	3.11	2.82	2.52	2.20	1.50	0.69	3.39	0.00
SPARC	SPARC	-1.99	3.57	3.42	3.12	2.74	1.85	0.81	3.57	0.049
MFD116	D5S209	0.88	0.78	0.68	0.58	0.48	0.31	0.15	0.88	0.00
IG83	D5S528	-3.61	2.73	2.85	2.72	2.47	1.73	0.79	2.87	0.091
IG52	D5S527	-4.33	2.03	2.17	2.06	1.84	1.20	0.47	2.17	0.095

Figure 2: *Photographs of individuals III.2 (A,B), III.11 (C,D) and II.3 (E,F).*

Figure 3: *Photographs of individuals III.6 (A,B), IV.4 (C,D) and IV.3 (E,F).*

8 cases (the remaining 2 markers, CSF1R and 116, being uninformative in the relevant meiosis, Figure 2 and 3). Conversley, individual II.1, who appears to be unaffected clinically and radiologically, also appears to be unaffected genetically as she does not inherit the affected allele with markers 2C7, SPARC, IG83 and IG52 (in the case of the remaining 4 markers it is not possible to dertermine which allele is inherited from the affected parent as both parents were deceased). Interestingly, individual IV.3, who appears to be unaffected both clinically and radiologically, but who is at risk of having inherited TCOF1, inherits the affected allele from his father with markers E5.12 and SPARC (and IG83), which closely flank the TCOF1 locus, suggesting that he is, in fact, affected.
Two individuals show recombination between the markers analysed and TCOF1. IV.4, who is clinically and radiologically affected, is recombinant with markers distal to TCOF1 (SPARC, IG83 and IG52), but non-recombinant with E5.12 which is proximal to TCOF1. Individual III.5, who is clinically and radiologically unaffected, is recombinant with IG83 and IG52, but non-recombinant with 2C7 and 2D10. This data is consistent with the previously reported location of the TCOF1 locus between IG90 and SPARC.[9,10]

Discussion

STRP markers exhibit hypervariability in the number of short tandem repeats which can be detected using the polymerase chain reaction (PCR).[15,16] A large number of these markers, unlike the majority of RFLPs, are highly polymorphic and therefore possess the advantage over "classical" markers that they make families more informative for linkage.[17] This is proving valuable in human genetic research, particularly in the study of rare Mendelian disorders where few families are available for study and pedigree structures are seldom ideal.
In the present investigation the high informativity of the markers used has permitted us to maximise the amount of linkage information that could be extracted from the family given that a number of individuals were unavailable for study. A relatively large number of STRPs have been isolated from the TCOF1 region and markers flanking the disease locus identified.[9,10,18,19] This marker framework permits the diagnostic evaluation of mildly affected individuals and apparently asymptomatic carriers in informative families and also enables first trimester prenatal diagnosis, using DNA-typing, to be performed. Currently prenatal diagnosis is only rarely possible using either ultrasound or fetoscopy in midtrimester.[20]
To date approximately 30 TCOF1 families have been investigated for linkage to markers in the region 5q31-34.[7,8,9,10] All the families that have been analysed support linkage of the disease locus to markers in the same region of the genome with none showing unequivocal evidence of nonlinkage, this data supports genetic homogeneity. Nevertheless, Treacher Collins syndrome has been associated with a number of different chromosomal anomalies raising the possibility that the disorder may be genetically heterogeneous. In one case TCOF1 has been associated with a cytogenetically balanced translocation t(6;16)(p21.31;p13.11), however, in this case the translocation was ultimately found not to cosegregate with the phenotype and the translocation breakpoints were excluded by linkage analysis.[7] TCOF1 has also been associat-

ed with a chromosomal deletion 4p15.32-p14, linkage analysis between TCOF1 and markers from the region of the deletion has also excluded the disorder from this region of the genome.[21] Moreover, the severe manifestations of this patient's phenotype may not have represented the condition present in patients with TCOF1. The third cytogenetic anomaly that has been reported to occur in association with TCOF1 is a balanced translocation t(5:13)(q11;p11) in a patient who also exhibited a significant decrease in the levels of the enzyme hexaminidase B, the gene for which is located at 5q11-13.[22] However, the TCOF1 locus has been excluded from close proximity to both of these breakpoints (Jabs and Dixon, unpublished). In this particular case the facial gestalt of the patient was inconsistent with a diagnosis of Treacher Collins syndrome.

While the gene mutated so as to cause TCOF1 occasionally appears nonpenetrant, in most cases where this is suspected, careful examination of the obligate carrier usually reveals minor stigmata of the syndrome.[5] Nevertheless, some individuals are so mildly affected that it is difficult to reach a diagnosis and thereby provide accurate genetic counseling. In the present study we have analysed 8 STRP markers for linkage to TOCF1 in a large family with multiple affected individuals. Linkage analysis suggested that TCOF1 in this family was linked to markers in the region 5q31-34, we have therefore and used the results to make diagnostic predictions in certain mildly affected and apparently unaffected individuals.

The results of the linkage analysis supported the affected diagnosis made in two individuals who exhibited only very minor stigmata of TCOF1, neither of whom had produced clinically affected offspring), and in individual III.10 (who has a clinically affected daughter, IV.4). Conversley, individual II.1, who appears to be unaffected clinically and radiologically, also appears to be unaffected genetically. More importantly, perhaps, individual IV.3, who appeared to be unaffected both clinically and radiologically, but who is at risk of having inherited TCOF1, appeared to be affected as assessed by linkage analysis.

While there is no apparent evidence of genetic heterogeneity in TCOF1, only a relatively small number of families have as yet been studied. Diagnostic predictions should therefore be undertaken with caution only in families showing significant evidence of linkage to 5q32-33.2 or when the possibility of genetic heterogeneity has been further minimised by the study of additional families.

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Summary and Conclusions

This thesis aims to contribute to the increasing clinico-genetic knowledge on syndromes with deafness or hearing impairment. If deafness is the only symptom, the patient is likely to be suffering from a hereditary non-syndromal abnormality. If other symptoms are also present, there is more likelihood of a syndromal form of hearing loss. All the autosomal dominant syndromes included in this thesis show morphogenic disruption of the branchial arches as a common characteristic. A non-syndromal form of hereditary deafness was also studied.
Branchiogenic disruption in these syndromes usually gives rise to partial or pure conductive hearing loss localised in the middle ear. One of the aims of the study was therefore to examine the extent to which reconstructive microsurgery on the middle ear or aural canal, or the fitting of a bone anchored hearing aid, can contribute to the rehabilitation of patients with syndromal hearing loss.
Depending on the available clinico-genetic knowledge on the disorders described in this thesis, some are described for the first time in the form of a syndrome, while others, such as the Treacher Collins syndrome, are presented from the point of view of a more sophisticated clinico-genetic syndrome. The latter is aimed primarily at the characteristics and penetrance of a particular disorder and consequently at the non-clinical presence in persons who, on the basis of their position in the family pedigree, are (potential) genetic carriers of an autosomal hereditary syndrome.
The thorough studies on the families affected by the syndromes described in this thesis could be supplemented by the initiation of gene-linkage studies in Nijmegen or abroad, or in the case of the Treacher Collins syndrome, could contribute to the ongoing gene-linkage studies in Manchester.

Chapter 1 describes a non-syndromal form of autosomal recessive hereditary hearing loss based on a unique pedigree. Due to the lack of any other stigmata, it is not always possible to establish the hereditary origin of this form of deafness. In such cases, the basis of an accurate diagnosis lies in the presence of other affected family members. If there are no indications of a hereditary cause, there is great likelihood that the patient is suffering from an autosomal recessive hereditary form of hearing loss. Data reported in the literature indicate that the incidence of non-syndromal autosomal recessive hereditary deafness or hearing impairment is 1:3000 to 4000 births.
Contrary to suggestions made by other authors, it was not possible to recognise carriers in this Dutch family with autosomal recessive deafness on the basis of very slight abnormalities in their audiograms. In another study performed in Nijmegen on autosomal recessive hereditary deafness, carrier detection proved to be impossible using clinical characteristics only.[1]

It is estimated that 5 or 10 genes are responsible for phenotypically similar forms of non-syndromal autosomal recessive hereditary deafness. In the near future, it may become possible to distinguish between some of these forms of hearing impairment and deafness on the basis of vestibular or radiological differences in affected persons using high tone audiometry in carriers. However, at present, gene-linkage studies seem to be the most promising approach to perfecting the diagnosis and detection of carriers. Unfortunately, there are very few detailed descriptions of such large families world-wide to meet the requirements necessary to perform gene-linkage studies. These families are generally isolates, such as described in Wallis, Switzerland, in Columbia or in Islamic countries with a higher consanguinity ratio. So far, we have not been able to localise the gene on one of the chromosomes using about 150 tested markers. This emphasises the need for further cooperation between institutes working in the same field.

Chapter 2 presents some of the results of studies on the Branchial-Oto-Renal (BOR) syndrome. The Department of ORL in Nijmegen came into contact with the Boys Town National Institute, Omaha, USA, for the first time in 1987. The families with the BOR syndrome who had previously been studied by Cremers et al. were re-evaluated and were found to be willing to participate in gene-linkage studies so that in the same year, blood samples could be sent to Omaha. It was not until June 1992 that this led to gene linkage. The translocation of the 8th chromosome found by coincidence elsewhere, quickly contributed to finding the required gene-linkage described in this chapter. The localisation of the gene responsible for the BOR syndrome on chromosome 8q has also been confirmed by others. This knowledge is extremely useful for other branchial arch syndromes which are difficult to distinguish clinically, such as the Branchio-Oto (BO) syndrome, the Branchio-Oto-Urethral (BOU) syndrome, Hemi-Facial-Microsomia (HFM) and the Branchio-Oculo-Facial (BOF) syndrome. In the literature, the BO syndrome, for instance, was described in which contrary to the BOR syndrome, no renal abnormalities were found. No convincing studies have been published which evaluated one complete family with more than 2 generations and failed to find any renal abnormalities in one or more affected persons with other characteristics. Therefore, for the past 10 years it has been assumed that the BO syndrome and the BOR syndrome are the same.
This chapter also contributes a new surgical technique for conductive hearing loss in the BOR syndrome. Teunissen (1992) demonstrated in his thesis that the severity of the malformation in congenital middle ear anomalies in clinically recognisable syndromes, is more complex than in other cases of congenital conductive hearing loss.[2] Development of new surgical techniques for such major middle ear anomalies is slow, partly because it is not possible to conduct relevant experimental research using animal models. The surgical techniques described in this chapter comprise the creation of a neo-oval window and myringo-chorda-vestibulopexy. They offer new possibilities for the treatment of these complex middle ear anomalies. Meanwhile, the new method of myringo-chorda-vestibulopexy has also been applied successfully to other patients at our clinic.

Chapter 3 describes a new syndrome which resembles the BOR syndrome (MIM # 120502). The characteristics of this syndrome are commissural lip pits, external ear

anomalies, pre-auricular sinus and conductive or mixed hearing loss. Although several of the symptoms are the same as those in the BOR syndrome, such as pre-auricular sinus (ear pits) and external ear anomalies, two important characteristics which are pathognomic for the BOR syndrome were lacking: cervical fistulae and renal abnormalities. Contrary to the BOR syndrome, the persons affected by the syndrome described in this chapter particularly demonstrated conductive non-progressive hearing loss without perceptive hearing loss, with very little variation between the affected persons. Furthermore, no abnormal vestibular function was found in any of the hearing impaired persons tested.
In view of the severity and nature of the hearing loss, 4 patients 6 ears underwent exploratory tympanotomy. Complex and widely-varying anomalies were found in the ossicular chain and unfortunately the results of reconstructive surgery were unsatisfactory.
Contrary to the BOR syndrome, no evidence was found of dysplasia of the inner ear on the CT scans of the ossa petrosa of the affected persons within this family. However, in one case, unilateral aplasia of the round window was suspected during exploratory tympanotomy, but CT examination of this ear demonstrated the presence of a round window which had been camouflaged by a bony plate. In such cases, any branchiogenic characteristics found in association with this syndrome should alert the otologist to the severity of the ossicular chain anomaly and the limited chance of successful reconstructive middle ear surgery.
Unpublished gene-linkage studies (Boys Town National Institute) have shown that no positive linkage could be achieved for this syndrome on chromosome 8q, the location of the BOR gene. Tests with other markers are currently under way, so the results could not be included in this thesis.

Chapter 4 describes another branchial arch syndrome, the Treacher Collins syndrome. It is currently assumed that ± 60% of the cases with this syndrome are caused by de novo mutations. Although there is wide variation in expression, non-penetrance has only been reported once in an abstract.
In the families described in this thesis, a number of persons appeared to have non-penetrant Treacher Collins syndrome (TCS). Subsequent radiological examination showed that being able to demonstrate the presence of zygoma aplasia or hypoplasia within the framework of family studies, made an important contribution to support of the diagnosis of whether or not a person was suffering from TCS. In this way it was possible to find additional clinical indications about the penetrance of TCS in the cases reported on by van Rijn and Cremers. The application of a simple Water's projection led to the diagnosis of TCS in several other persons mentioned in these reports. Gene-linkage studies detected a further 3 persons without any clinical characteristics of TCS as being carriers of the TCS gene with phenotypical non-penetrance. There are no other studies in the literature in which families with 3 generations underwent audiological, vestibular, radiological and genetic examination. The existence of non-penetrance of TCS has not been demonstrated convincingly in the past. Therefore, it is unlikely that 60% of the isolated cases of TCS are caused by de novo mutation. Detailed evaluation of more than one or two family members is usually lacking. By using new markers on the Dutch families, it was possible to make a more accurate localisation of the gene on 5q32-33.2. Further research with the aim of cloning is currently under way.

This chapter also discusses the possibility of reconstructive ear surgery for patients with this syndrome. So far, the results of ear surgery in patients with TCS with congenital conductive hearing loss have been poorer than those achieved in general.
The branchiogenic syndromal diagnosis forms an indication of the severity of the malformations which can be expected in the middle ear. In patients with TCS, there is wide variation in the severity of the congenital anomalies which may be present in the auricle, aural canal and/or the middle ear.
In this thesis, the patients were divided into categories such as major and minor anomalies on the basis of clearly defined classifications and subclassifications. From an overview of the results of ear surgery on patients with TCS in the literature and from our own experience, we could conclude that particularly patients with TCS and congenital aural atresia do not form suitable candidates for reconstructive surgery because the results have generally been disappointing. Adequate, long-term improvement in the patient's hearing has only been achieved once in the case of type IIA aural atresia. However, type IIA aural atresia is rare in the TCS. If aural atresia is present, it is usually a type III anomaly. The application of this subclassification for aural atresia was once again useful to make a preoperative prediction of the chance of successful reconstructive surgery of the aural canal.
In these patients, the bone-anchored hearing aid has proved to be a good alternative if a conventional bone conduction hearing aid cannot be fitted for anatomical or cosmetic reasons.[3]
Patients whose hearing loss is caused purely by a middle ear anomaly can be treated successfully with reconstructive ear surgery. There is wide variation in middle ear pathology. Complex anomalies, such as a monopodal stapes and/or the facial nerve overhanging the oval window, are not infrequent. Fixation of the stapes suprastructure to the bony canal of the facial nerve in the case of a mobile footplate is also seen fairly often. Dysplasia of the oval window and the absence of the stapes and incus can also occur; the neo-oval window technique and the interpositioning of a malleo-vestibulopexy, or myringo-chorda-vestibulopexy are among the surgical possibilities for these patients.

This study has shown that although our knowledge on hereditary deafness has increased considerably over the past 25 years, it is still possible to further expand our detailed clinical and genetic knowledge on these forms of deafness and hearing impairment. The results have direct meaning for clinical practice. This thesis makes a contribution to our knowledge on four such syndromes. Ongoing and future clinico-genetic research is expected to improve the diagnosis of carriers and affected persons and provide more insight into the pathogenesis.
It is also of importance to continue improving the surgical techniques and rehabilitation of hearing impairment. This study has shown that fitting the percutaneous bone-anchored hearing aid to patients with TCS and major bilateral aural atresia is a new and useful procedure.
If surgical intervention is likely to improve a patient's hearing, it is worthwhile to refer the patient to a specialised centre owing to the wide variation in pathology and the rarity of such syndromal anomalies.

References

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nimale afwijkingen in hun audiogram. Ook in ander Nijmeegs onderzoek waarin ook autosomaal recessief overervende niet syndromale doofheid bestudeerd is, is carrier detectie op basis van klinische kenmerken niet gelukt.[1] Het aantal verantwoordelijke genen voor phenotypisch gelijke vormen van nonsyndromal autosomal recessive inherited deafness wordt geschat op 5 tot 10. Mogelijk kunnen in de nabije toekomst sommige van deze vormen van slechthorendheid en doofheid toch van elkaar onderscheiden worden op basis van bijvoorbeeld vestibulaire of radiologische verschillen bij de lijders of op basis van hoge tonen audiometrie bij carriers. Genkoppelingsstudies lijken echter op dit moment de meest aangewezen weg om de diagnostiek te vervolmaken en om tot carrierdetectie te kunnen komen. Helaas zijn er wereldwijd slechts enkele soortgelijke grote families gedetailleerd beschreven om te voldoen aan de eisen die een genkoppelingsstudie mogelijk maken. Het gaat dan vooral om isolaten zoals in Wallis, Zwitserland, in Columbia of in Islamitische landen met een hogere consanguiniteits ratio. Bij de hier beschreven familie is het tot op heden nog niet gelukt het gen met behulp van de ongeveer 150 geteste markers op een van de chromosomen te lokaliseren. Het benadrukt de wenselijkheid tot verdere samenwerking tussen de op dit terrein werkzame instituten.

In **Hoofdstuk 2** worden enkele onderzoeksresultaten bij het branchio-oto-renaal (BOR)syndroom beschreven. In 1987 werden de eerste kontakten gelegd tussen het Boys Town National Institute, Omaha, USA en the Department of ORL in Nijmegen, the Netherlands. De families met het BOR syndrome die eerder door Cremers en medewerkers waren onderzocht werden opnieuw beoordeeld en deze families werden bereid gevonden mee te werken aan genkoppelingsstudies zodat nog in dat zelfde jaar bloedmonsters naar Omaha gebracht werden. Het duurde tot juni 1992 voordat dit tot genkoppeling leidde. Een elders toevallig gevonden translocatie van het 8ste chromosoom droeg snel bij tot het vinden van de gewenste genkoppeling, zoals in hoofdstuk 2 wordt beschreven. De lokalisatie van het gen verantwoordelijk voor het BOR syndroom op chromosoom 8q is ook door anderen bevestigd. Deze kennis is zeker ook nuttig om differentiatie tussen andere klinisch moeilijk te onderscheiden kieuwboog syndromen zoals het Branchio-Oto syndroom (BO), het Branchio-Oto-Urethral syndroom (BOU), de Hemi-Facial-Microsomia (HFM) en het Branchio-Oculo-Facial syndroom (BOF) mogelijk te maken. In de literatuur wordt bijvoorbeeld het BO syndroom beschreven waarbij in tegenstelling tot het BOR syndroom geen nierafwijkingen gevonden worden. Omdat er geen overtuigende studies bekend zijn waarbij in één gehele familie met meer dan 2 onderzochte generaties nierafwijkingen ontbraken bij een of meer van de lijders met andere kenmerken wordt de laatste 10 jaar op deze klinische gronden aangenomen dat het BO en BOR syndroom gelijk zijn.
In dit hoofdstuk wordt ook een bijdrage geleverd over een nieuwe operatieve behandeling van de geleidingsslechthorendheid bij het BOR syndroom. Teunissen (1992) heeft in zijn thesis aangetoond dat de ernst van de misvorming van congenitale middenooranomaliën bij klinisch herkenbare syndromen meer complex is dan voor de overige gevallen van congenitale middenoorslechthorendheid.[2] Het ontwikkelen van nieuwe operatieve technieken voor dergelijke ernstige middenoor anomaliën verloopt langzaam ook al omdat relevant experimenteel onderzoek in diermodellen onmogelijk is. De in hoofdstuk 2 beschreven chirurgische technieken met het aanleggen van een neo-oval window en de myringo-chorda-vestibulopexie openen nieuwe mo-

gelijkheden voor de behandeling van deze complexe middenoorproblematiek. Inmiddels is deze nieuwe methode van de myringo-chorda-vestibulopexie ook al bij andere patiënten succesvol toegepast.

In **Hoofdstuk 3** wordt een op het BOR syndroom gelijkend nieuw syndroom beschreven (MIM # 120502). De kenmerken van dit syndroom zijn commissural lippits, oorschelp afwijkingen, pre-auriculaire sinus en conductieve of gemengde slechthorendheid. Hoewel het enkele kenmerken met het BOR syndroom gemeen heeft, zoals de pre-auriculaire sinus (earpits) en de oorschelpafwijking, ontbreken 2 belangrijke kenmerken die juist pathognomonisch zijn voor het BOR syndroom: cervicale fistulae en nierafwijkingen. In tegenstelling tot het BOR syndroom bleek dat bij de lijders van het in hoofdstuk 3 beschreven syndroom met name sprake was van een conductief niet progressief gehoorverlies zonder een binnenoorverlies. De variatie hiervan bleek gering te zijn. Evenmin werd er bij enkele geteste lijders met een gehoorverlies een abnormale vestibulaire functie vastgesteld. Gezien de ernst en de aard van het gehoorverlies werden bij 4 patiënten (6 oren) één of meer middenoor inspecties verricht. De afwijkingen aan de gehoorbeenketen bleken divers en complex te zijn en de resultaten van gehoorverbeterende chirurgie waren helaas niet bevredigend. In tegenstelling tot het BOR syndroom werden er bij CT-scanning van de ossa petrosa bij de lijders binnen deze familie geen aanwijzingen gevonden voor het bestaan van een dysplasie van het binnenoor. Wel werd in een casus een enkelzijdige aplasie van het ronde venster geconstateerd tijdens een middenoor inspectie. Een CT-onderzoek van dat oor leerde dat dit berustte op een benige plaat die het aanwezige ronde venster camoufleerde. Ook hier dient de aanwezigheid van branchiogene kenmerken van dit syndroom de otoloog te waarschuwen voor de ernst van de ketenanomalie en daarmee op een kleinere kans op succesvolle reconstructieve middenoorchirurgie. Inmiddels is uit nog niet gepubliceerde genkoppelingsstudies (Boys Town National Institute) gebleken dat er voor dit syndroom geen positieve koppeling bereikt kon worden op chromosoom 8q, de locatie van het BOR-gen. Het testen van andere markers is nog in volle gang zodat de resultaten hiervan niet meer in deze thesis konden worden opgenomen.

In **Hoofdstuk 4** wordt een ander kieuwboogsyndroom, het Treacher Collins syndroom, beschreven. Naar tot nu toe wordt aangenomen berust $\pm$ 60% van de gevallen bij dit syndroom op de novo mutaties. Hoewel er zeker een aanzienlijke variatie in expressie bestaat, is non-penetrantie slechts eenmaal in een abstract gerapporteerd. Bij de in deze dissertatie beschreven families bleken er ogenschijnlijk diverse personen te bestaan met een non-penetrant Treacher Collins Syndroom (TCS). Aanvullende radiologische diagnostiek leerde echter dat het aantonen van het bestaan van een zygoma-aplasie of hypoplasie in het kader van de familiestudie een belangrijke bijdrage was ter ondersteuning van de diagnostiek van het al dan niet lijder zijn aan dit Treacher Collins Syndroom. Aldus konden in het door van Rijn en Cremers gerapporteerde geval alsnog klinische aanwijzingen verkregen worden voor penetrantie van het Treacher Collins syndroom. Door toepassing van een eenvoudige Water's projectie werd alsnog de diagnose TCS gesteld bij enkele personen in deze studies. Door genkoppelingsstudies werden aanvullend nog 3 andere personen zonder enig klinisch kenmerk van het Treacher Collins syndroom herkend als dragers van het TCS-

gen met phenotypisch non-penetrantie. In de literatuur zijn géén andere studies gevonden waarbij families van 3 generaties zowel audiologisch, vestibulair, radiologisch als genetisch onderzocht zijn. Het bestaan van non-penetrantie is wat betreft TCS nooit eerder overtuigend aangetoond. Daarom valt te betwijfelen of inderdaad bij 60% van de geisoleerde gevallen van TCS syndroom sprake is van een de novo mutatie. Gedetailleerd onderzoek van meerdere familieleden ontbreekt namelijk meestal.
Het bleek tevens mogelijk te zijn door nieuwe markers bij de Nederlandse families te gebruiken het gen nauwkeuriger te localiseren op 5q32-33.2. Verder onderzoek met als doel clonering van het gen is gaande.
In hoofdstuk 4 wordt evenzo uitvoerig stilgestaan bij de mogelijkheid van reconstructieve oorchirurgie bij dit syndroom. Ook voor de oorchirurgie bij patiënten met het Treacher Collins syndroom vanwege een aangeboren middenoorslechthorendheid geldt dat de resultaten niet zo gunstig zijn als doorgaans. De branchiogene syndromale diagnose blijkt ook hier een indicatie over de te verwachten ernst van de misvorming van het middenoor. Bij patiënten met het TCS betaat een grote variatie in de ernst van een mogelijke aanwezige aangeboren anomalie van de oorschelp, gehoorgang en/of het middenoor, die in deze thesis volgens duidelijk omschreven classificaties en subclassificaties onderscheiden worden onder meer in major en minor anomalies. Door het verschafte overzicht van de resultaten van oorchirurgie bij TCS in de literatuur en door het geven van de eigen ervaring bij patiënten van het AZN kan worden geconcludeerd dat met name patiënten met TCS en een gehoorgangatresie een weinig aantrekkelijke groep zijn voor reconstructieve chirurgie, omdat deze resultaten over het algemeen teleurstellen. Slechts eenmaal gelukte het in het geval van een IIA gehoorgangatresie een voldoende goede duurzame verbetering van het gehoor te bereiken. Een type IIA gehoorgangatresie blijkt bij TCS echter een zeldzaamheid. Als er sprake is van een gehoorgangatresie betreft het meestal een type III anomalie. Het toepassen van deze subclassificatie voor gehoorgangatresiën toont bij deze groep patiënten opnieuw zijn waarde, om preoperatief de kans op succesvolle reconstructieve chirurgie van de gehoorgang te kunnen voorspellen. Het Bone Anchored Hearing Aid blijkt een goed alternatief juist voor deze patiënten, waarbij de aanpassing van een conventionele beengeleider om anatomische en cosmetische redenen niet mogelijk is.[3]
Patiënten waarbij het gehoorverlies berust op een uitsluitend in het middenoor voorkomende anomalie kunnen wel geholpen worden met reconstructieve oorchirurgie. De variatie in middenoorpathologie is groot waarbij complexe afwijkingen zoals een monopodale stapes en een over het ovale venster overhangende nervus VII tamelijke frequente afwijkingen zijn. Opvallend is ook de fixatie van het stapeskopje aan het benige kanaal van de nervus facialis bij een op zich mobiele stapesvoetplaat. Een dysplasie van het ovale venster met een ontbreken van een stapes en incus komt ook voor, zodat de technieken van een neo-ovale venster, en als interpositie een malleovestibulopexie of een myringo-chorda-vestibulopexie tot de mogelijkheden behoren.

Uit de voorgaande hoofdstukken is gebleken dat, alhoewel de kennis over erfelijke doofheid met name de laatste 25 jaar al fors is toegenomen, het nog steeds mogelijk is onze gedetailleerde klinische en genetische kennis over deze vormen van doofheid en slechthorendheid verder uit te breiden. De verkregen resultaten hebben direct be-

tekenis voor de klinische patiëntenzorg. De hier gepresenteerde dissertatie levert daaraan voor een viertal van deze ziektebeelden een eigen bijdrage. Huidige en toekomstige resultaten van het klinisch-genetisch onderzoek zullen een verdere verbetering van diagnostische mogelijkheden voor de lijders en de carriers moeten brengen. Ook kan hierdoor het inzicht in de pathogenese vergroot worden.
Het blijft van belang om de operatieve mogelijkheden van revalidatie van gehoorverliezen verder te verbeteren. Toepassing van de percutane Bone Anchored Hearing Aid blijkt zoals in deze dissertatie beschreven voor de patiënten met TCS en een ernstige dubbelzijdige gehoorgangatresie al een nieuwe en nuttige aanwinst. De myringochorda-vestibulopexie is een ander voorbeeld. Wanneer een operatieve verbetering van het gehoor tot de mogelijkheden behoort lijkt het nuttig om vanwege de grote verscheidenheid aan pathologie en de zeldzaamheid van deze pathologie deze behandeling over te laten aan daarin gespecialiseerde centra.

Referenties

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De letters *co-pro* op pagina IV zouden tussen haakjes moeten staan.
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Noortje ..... (gecensureerd door Noortje zelf).

De correcties van en vertalingen in het Engels werden verricht door mevrouw J. Abma-Hill.

Curriculum vitae

Na het middelbare onderwijs (Atheneum B) in Nijmegen genoten te hebben volgde aldaar de Medische Studie aan de Katholieke Universiteit. Het artsexamen werd behaald in november 1987. Tijdens de studie werd als student-assistent onderzoekservaring opgedaan bij de afdeling Pathologische Anatomie (1984-1985) en de afdelingen Anaesthesiologie en Interne Geneeskunde (1985). Na de medische studie werden de eerste schreden gezet binnen de afdeling Keel-, Neus- en Oorheelkunde van het Academisch Ziekenhuis Nijmegen als onderzoeks-assistent.
De opleiding tot keel-, neus- en oorarts ving in feite aan op 1 augustus 1988 te Liverpool, Engeland. Tijdens de verdere opleiding in Nijmegen (1 september 1989 - 1 maart 1994) werd stage gelopen in het Rijnstate Ziekenhuis te Arnhem en het Antoni van Leeuwenhoekhuis te Amsterdam.
Sinds 1 maart 1994 is H.A.M. Marres werkzaam als staflid in het Instituut voor Keel-, Neus- en Oorheelkunde van het Academisch Ziekenhuis te Nijmegen.